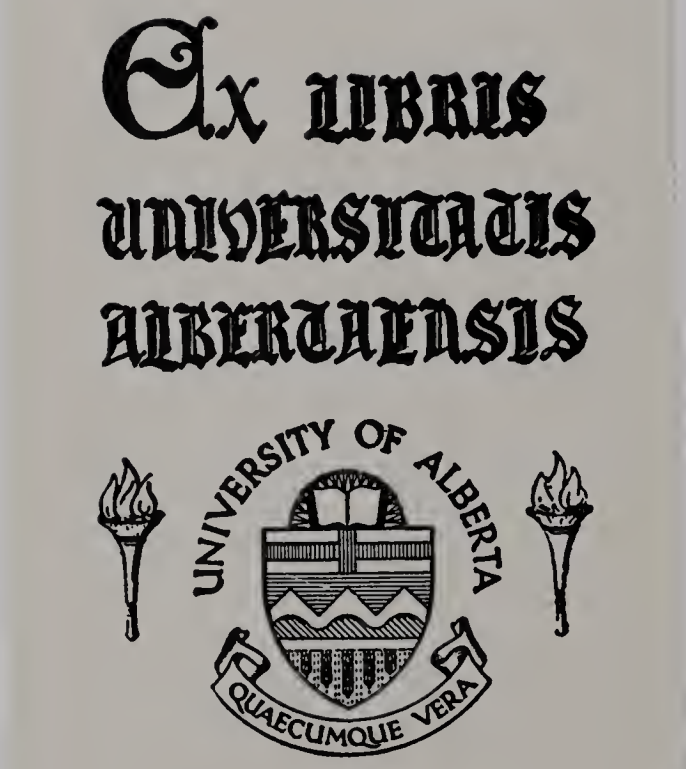

University of Alberta
Printing Department

THE UNIVERSITY OF ALBERTA

THE CONTROL OF THE MELANOPHORES IN THE BACKGROUND COLOUR RESPONSE OF *FUNDULUS HETEROCLITUS*

by

FRANK S. ABBOTT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

MAY 1966

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The control of the melanophores in the background colour response of Fundulus heteroclitus," submitted by Frank S. Abbott in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The melanophores of most teleost fish exhibiting a response to the background are under the control of the autonomic nervous system. There is general agreement that the sympathetic division of the autonomic nervous system supplies aggregating fibres to the melanophore, but conclusive evidence is lacking for the participation of the parasympathetic division in melanophore control. The experimental approach used in this study had three major goals: (a) to determine the effects and sites of action in Fundulus of agents known to affect the autonomic nervous system in mammals; (b) to determine, through the use of these agents, whether one or both divisions of the autonomic nervous system were involved in colour change; and, (c) by testing a variety of physiologically active substances, to determine the specificity of the melanophore response, and to find whether it is sensitive to hormones and other agents which influence melanophores in other animals.

The responses of the melanophores of Fundulus heteroclitus to a number of agents were studied in vivo by injection experiments and in vitro on isolated scales. Additional tests of some of these substances were carried out on fish with fresh and faded caudal bands. These preparations have, respectively, fresh and chronically denervated melanophores.

Sympathomimetic drugs caused aggregation of melanin in melanophores under all circumstances. Alpha-adrenergic blocking agents caused dispersion. Propanalol, a β-blocking agent, had no effect. These results are taken as confirming the presence of an adrenergic mechanism for aggregation.

All parasympathomimetic agents tested had no effect. Atropine in large doses brought about a slow but persistent dispersion in vivo, but

had no effect *in vitro*. These results support the opinion that there is no direct cholinergic mechanism involved in colour change in *Fundulus*.

Attempts to stimulate ganglia with nicotine resulted in transient aggregation *in vivo*; *in vitro* nicotine caused dispersion; both effects were observed only at high concentrations. Lower concentrations had no effect.

Ganglion blocking agents evoked dispersion of varying persistence *in vivo*. Pentolinium caused dispersion *in vitro* while others had no effect. Hexamethonium prevented the formation of fresh caudal bands but caused dispersion in faded caudal bands, as did presidal, another ganglion blocking agent.

The actions of postganglionic sympathetic fibre blocking agents were not consistent. Bretylium caused dispersion *in vivo* at high dose levels and aggregation at low doses. It was ineffective *in vitro* but caused dispersion in caudal bands. Guanethidine, a similar drug, caused aggregation *in vivo* at high doses.

Two monoamine oxidase inhibitors, parnate and pargyline, were tested. Parnate caused aggregation *in vivo*, inhibited adaptation to a black background and caused dispersion *in vitro*. Pargyline had no effect. The effect of parnate is probably similar to its known action of preventing the destruction of catechol amines in mammals.

The actions of a group of biogenic substances, other than the chemical transmitters, were observed. Serotonin caused aggregation *in vivo* and *in vitro*. Melatonin and posterior pituitary gland powder were ineffective. As the *in vivo* tests were carried out on intact fish, these results may be regarded only as suggesting that hormonal control of colour change in *Fundulus*, if present, is of lesser importance than nervous control.

While a number of substances were shown to have aggregating effects *in vivo*, attempts to block this action by the prior injection of an appropriate blocking agent yielded inconsistent results. It is possible that

this inconsistency may be related to technical difficulties in administering the agents.

All the evidence presented indicates that in physiological colour change in Fundulus the melanophores are controlled by an adrenergic mechanism; that there is no cholinergic mechanism; and that hormones are probably of minor importance in this phenomenon.

Some evolutionary and ecological implications of the phenomenon of colour change in Fundulus and in other cold-blooded vertebrates are discussed.

ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. D.M. Ross, Dean of Science, for suggesting this research, and for his interest and encouragement during the study. Thanks are also due members of the Department of Zoology, both academic and technical staff, whose suggestions and assistance helped greatly. I thank Dr. C.W. Nash of the Department of Pharmacology, whose help in the form of gifts of drugs and discussions, as well as criticism of the manuscript was invaluable. Dr. Daniel of the same Department also donated samples of drugs for testing.

I am particularly grateful to Dr. Frank Schwartz, Curator of Fish, Chesapeake Biological Laboratory, Solomons, Maryland, who provided the fish used in the study. Without his participation the research would have been impossible.

I thank my wife, Joyce, whose forbearance, encouragement, and assistance in preparing this thesis made the task much easier than I had anticipated.

This study was supported by grants from the National Research Council of Canada to Dr. Ross, and by the award of a research assistantship by the University of Alberta. This assistance is gratefully acknowledged.

TABLE OF CONTENTS

Table of Contents cont.

LIST OF TABLES

I. INTRODUCTION

Scientific interest in the phenomenon of adaptation to background has been expressed in publications for well over a century (Parker 1948). These investigations have been summarized in several monographs and reviews, notably those by Parker (1948), Waring (1963), and Fingerman (1965).

Autonomic nervous control of melanophore in teleosts was clearly shown by Pouchet (1871-76) and this means of control was believed to be universal for over 40 years. Spaeth (1913) and Wyman (1924) demonstrated that hormones also played a part in chromatic behaviour in Fundulus.

As work on these two concepts of control went forward it became apparent that both means of control of the melanophore might be present in a given species, and that the relative importance of each varied from species to species.

In the case of Fundulus heteroclitus two hypotheses concerning physiological colour change have been proposed. The first, developed by Parker and others, postulates dual innervation of the melanophore by the autonomic nervous system. The second, developed by Hogben (1924) and maintained by Waring involves single innervation by aggregating fibres of the melanophore interacting with hormones, and relying to a considerable extent on the time relations of physiological colour change under various circumstances.

These two conclusions arose from research which employed the customary methods of surgery, histology, anatomy, and variation of environmental factors. One might think that traditional methods of investigating melanophore control in Fundulus had been exploited as fully as possible. Yet the uncertainty about the results and interpretations of some drug experiments

suggested that certain earlier work might be repeated and extended with standardized procedures. Moreover, new agents acting upon the autonomic nervous system and its effectors became available, and it was decided to try to elucidate the means of control of physiological colour change in Fundulus using some of these as well as some of the older drugs. The program of research was influenced to some extent by the fact that Fundulus heteroclitus is not native to Alberta and the source of supply was a laboratory on the Atlantic Coast of the United States. This made it imperative to attack the problem by ways which would be as sparing of fish as possible.

In the last century some studies were made on the effects of drugs on fish melanophores (Pouchet 1871a, c) but these were unsystematic and relatively unfruitful, probably because the number of drugs available was small and knowledge of their site and mode of action largely lacking.

The discoveries in the twentieth century out of which arose the theory of chemical transmitters at the nerve endings, associated with the names of Dale and Loewi, opened up new possibilities for interpreting drug effects and at the same time provided a coherent theory of the mechanism of nervous control over effector cells. This approach found many applications in the study of melanophore control.

Among the earlier publications on drug effects on fish melanophores, those of Spaeth (1916a), Spaeth and Barbour (1917a, b), Wyman (1924b), Gilson (1926), Smith (1931), and Osborn (1939) should be mentioned. The agents were administered by injection and immersion, and their effects on the melanophores of isolated scales were studied as well.

The results, as epitomized in the monographs by Parker and Waring, seemed to leave the question of melanophore control in Fundulus undecided.

More recently, Wilber (1954, 1958, 1960) studied melanophore responses in Fundulus to octamethyl phosphoramide (OMPA), hexamethonium, decamethonium and lysergic acid. Turner and Carl (1955) investigated the effect of reserpine, using Betta splendens. They concluded that in this species reserpine acts directly on the melanophore causing dispersion. The dispersing effect of the same drug on melanophores of Rana was reported by Khazan and Sulman (1961). A comparative study of the effects of psychoactive drugs on the melanocytes of fish and frogs was made by Scott (1962), and the pharmacology of poikilotherms was well covered by Fänge's (1962) review. The systematic study of nervous control of chromatophores in teleosts by Pye (1964), and his conclusions, has important implications in the investigation of melanophore responses in Fundulus. Healey and Ross (in press) carried out an extensive survey of the actions of drugs in vivo, in vitro, and in operated Phoxinus using certain indices for comparing the effects. Their experimental approach was similar to that reported here.

The basis for the classification of the agents acting upon the autonomic nervous system and its effectors used in this study is one of convenience. It was thought that terms such as sympathomimetic, adrenolytic, anticholinergic, while indicating the nature of the effect gave no suggestion of the site or mode of action of the agent bringing about the effect. Furthermore, it is difficult to classify rationally agents such as reserpine, amphetamine, and the monoamine oxidase inhibitors (MAOI) under such headings, because these, and certain other drugs, have a diversity of actions, many still not clearly understood. Thus, while a drug which interrupts an adrenergic mechanism at any level may be termed anti-adrenergic, it was felt that a more specific terminology incorporating existing knowledge of the site and mode of

action of drugs in other animals might well shed more light on their effect on the chromatic control mechanism in Fundulus.

It is recognized, however, that drug effects in fishes and mammals may not be similar (Pye 1964). Nevertheless, in the absence of conclusive evidence that the chemical mediators of the autonomic nervous system in fish are different to those in mammals, it seems justified to assume, tentatively, that they are very similar if not identical. Strictly speaking the presence and function of chemical mediators can only be demonstrated by their detection in tissue extracts obtained under appropriate conditions of excitation. Such demonstrations are difficult to provide in many cases however and in such cases most investigators make the assumption that is made here.

In sum, it is clear that despite the vast amount of work on teleost colour change, and on Fundulus heteroclitus in particular, there are still a number of outstanding problems. In this investigation it was intended to carry out a comprehensive, standardized survey of drug effects on the melanophores of Fundulus in order to: 1) test for the existence of a cholinergic mechanism; 2) gain information on the physiological state of caudal band preparations; 3) gain further information on the properties of the cholinergic aggregating mechanism by the use of various blocking agents.

It is perhaps scarcely necessary to note that integrity of the visual system is an essential condition for the occurrence of physiological colour change in Fundulus. The change is initiated by perception of the light stimulus at the retina and this information is relayed through the central nervous system to the effectors. It is the mechanism of the response of the effectors which has been the primary focus of interest in this work.

II. MATERIALS and METHODS

1. Fish handling and care

Fundulus heteroclitus L. used in this study were obtained from Chesapeake Bay, Maryland. Shipments were made at all seasons. Both males and females were used as the more brilliant colouring of the male fades under laboratory conditions. Fish under three inches were not suitable because they did not tolerate handling well and the risk of leakage after injection was greater.

Details of preparation and shipment of the fish are as follows (Schwartz 1966). The fish were held in aquaria with running brackish water. When the air temperature was above 40° C the fish were placed in plastic bags containing 40 to 60 each, about 12 hours before shipment. Just prior to shipment the water was changed, the bag was placed within another plastic bag and oxygen bubbled in for about one minute. The air space above the water was filled with oxygen for about 30 seconds. Both bags were sealed by twisting the neck and closing with rubber bands. Ice was placed within or adjacent to the double plastic bags. The bags were placed in polythene foam boxes, which were taped shut and placed in cardboard cartons. The cartons were sealed and taken to an airport, a drive of about 80 minutes, and remained at the airport for at least two hours before being loaded.

If the temperature was below 40° C the ice was omitted.

The procedure outlined above was reasonably satisfactory. Only 25 of 646 fish shipped by this method were dead on arrival.

Later a variation in procedure was introduced which proved even more satisfactory. The Fundulus were placed in metal tubs 3/4 full of water,

about 50 to 60 to a tub, and the temperature brought to 1.5° C in about 12 to 24 hours. The fish became stuporous. On the following morning the fish were put in bags as before, water at 1.5° C added and oxygen bubbled in. The sealed bags were packed as noted above.

The latter procedure reduced losses in transit to five of approximately 1,100 fish shipped. Losses due to damage to the cartons and boxes occurred, but if the cases were intact the fish usually travelled in good condition. The oxygen bubbled in before sealing the bags was adequate for at least 72 hours.

On arrival the bags were emptied into a 40-gallon tank and brought to 15° C and fresh, dechlorinated water over a period of four hours. Dead and seriously injured fish were removed at this time. The fish were then given two prophylactic treatments. The first consisted of adding hyamine hydrochloride to the water to give a concentration of about 1:75,000. At the first sign of distress the disinfectant solution was replaced by fresh dechlorinated water. After one to two hours the fish, in batches of about 20, were given a bath in 1% malachite green solution for not more than 30 seconds, a quick rinse, and placed in aquaria at a density of about 1.5 fish per gallon. The duration of the treatments was varied as dictated by the condition of the fish.

At first the fish were kept in dechlorinated water in aquaria equipped with air lift filters. Although the prophylaxis noted was effective for a time, Saprolegnia and other infections eventually appeared. Infected fish were segregated daily and subjected to the following daily treatment: one hour in a bath of pyridyl mercuric acetate 1:50,000; 10 to 20 minutes in brine at twice sea water concentration; up to five minutes in a bath of malachite green 0.75%. For chronic treatment the fish were kept in water to which acriflavine had been added until a strong yellow-green colour developed.

The treatment outlined above reduced mortality about 50%, but morbidity remained high. To reduce morbidity, after treatment on arrival the fish were placed in 25% sea water (using Neptune Salts). While fungus infections still appeared occasionally, the decimating epidemics experienced when the fish were in fresh water were absent. Prophylaxis, rigorous segregation and treatment of diseased fish, and the other measures noted, reduced losses from disease by 50%, decreased the incidence of fungus infection drastically, and appeared to eliminate almost completely bacterial and parasitic diseases. Losses from disease eventually levelled off at about 1-2% of the stock per week. After successful treatment the fish were allowed to recover for several weeks before being used for experiments.

Fish were fed three times a week on a small-sized commercial fish food, except after arrival when at least 48 hours elapsed before food was offered.

2. Experiments with intact fish

Four fish were taken from the stock and adapted in pairs for at least 15 minutes to black and white backgrounds in pans 31 x 28 x 13 cm and 35 x 30 x 13 cm respectively. The water depth was 7 cm. Normally Fundulus complete the colour change in less than five minutes, so that adaptation time was more than adequate for completion. As far as possible unused fish were selected, but if previously used fish were employed, the date and type of treatment were recorded. In any case more than three weeks elapsed between experiments on the same fish.

At the end of the background adaptation period the fish were compared to a series of grey tinted papers of the Ostwald type* and the nearest match recorded. For closer comparison tints intermediate between

* See photographic reproduction in Appendix III.

the integers of the Ostwald Scale were recorded as 0.5 more or less than the nearest Ostwald number. Strips of the tinted papers were sealed between microscope slides and held beside the fish to facilitate comparison. The details of recording are given on page 13 and Appendix II.

The fish were then given an intraperitoneal injection of the agent under investigation. Initially a standard dose of 100 micrograms (mcg.) in 0.1 ml was given. In all experiments the volume of the injection was 0.1 ml but the dose was varied in accordance with the results of the initial injections. The site of injection was a few millimetres anterior to the vent and slightly lateral to the midline, in order to avoid injuring the subintestinal blood vessels. The fish were caught and held in a piece of nylon netting about seven inches square placed over the hand. After injection the site was observed for a few moments in an attempt to detect leakage before returning the fish to the appropriate background.

Five minutes after injection comparison with the tinted papers was made and recorded, and repeated at five-minute intervals for 30 minutes.

The Fundulus were then placed in numbered and perforated clear plastic boxes, 11 x 11 x 3.5 cm. If it was desired to note the effect of the injection on ability to adapt to a different background, or if the effect was not clear, background exchanges could then be carried out conveniently. The results of background exchanges are shown under the heading Adaptation in Table 1.

At the end of the experiment the fish in the boxes were placed in a tank overnight, to allow lethal, delayed or persistent effects to become apparent. The next morning survivors were given a short bath in a malachite green solution, segregated on the basis of the agent injected and returned to holding tanks.

Illumination was by overhead incandescent bulbs giving an intensity of 25-foot candles at the surface of the water covering the backgrounds. Light intensity was measured using a Universal Photovolt Model No. 200 light meter.

Drugs were made up to the appropriate concentration in a saline of the following composition: 6 parts 0.1M NaCl, 1 part 0.1M KCl, 0.35 parts 0.1M $CaCl_2$ (Spaeth and Barbour 1917a). The volume injected was 0.1 ml. Control injections of the same volume of saline alone were also made. This method makes separate controls unnecessary as each fish acts as its own control. For brevity these experiments are referred to as in vivo. The results are shown in Table 1.

3. Experiments using isolated scales

The drugs were dissolved in the saline mentioned above to give a concentration of 1 mg per ml, and the control scales were also placed in this saline. Three scales were removed and each was placed in saline in a small glass dish on the stage of a binocular zoom dissecting microscope. Mean melanophore indices were recorded for both the experimental and the control scales in Spaeth's saline until a constant mean melanophore index (M.M.I.) had been obtained for at least 10 minutes. The M.M.I. was calculated by assigning each of 10 melanophores selected for observation under the appropriate number of the Melanophore Index as introduced by Hogben and Slome (1931), for Amphibia and applied to teleosts by Healey (1951) (Fig. 1), multiplying the number of melanophores by the Melanophore Indices and dividing the total of these products by 10. The saline in the experimental chambers was then replaced by a similar volume of the drug solution, and the saline in the control chamber was replaced with fresh saline at the same time. M.M.I. readings were taken at five-minute intervals following this for at least 30 minutes.

In most instances, and especially if the drug effect was not particularly marked, the melanophore response was checked by replacing the saline of the control chamber with the drug solution at the end of the period mentioned above. The results are shown in Table 3.

To determine the effect of light at room temperature (23°-24° C) the scale was placed in a glass chamber containing saline, on the insulated and fan-cooled stage of the microscope. The chamber was illuminated from below throughout the observation period. Tests were made prior to the experiments to insure that the stage did not become heated. The control was a scale in saline at room temperature, illuminated only long enough to record the M.M.I.

To detect the effect of heat, the experimental chamber was placed on the stage of a microscope which had been masked with a sheet of thin, opaque plastic film. Tests made prior to the experiments showed that when continuously illuminated from below the masked stage reached a maximum temperature of 32.5° C in about 75 minutes. The control was a scale in saline at room temperature (25°-26° C) which was illuminated only long enough to record the M.M.I., as was the experimental chamber.

The effect of cold was tested by placing the experimental chamber on melting ice between readings. It was brightly illuminated from below when readings were made. The control was a scale in saline at room temperature, again illuminated for a very short period (about 10 seconds) for readings at five-minute intervals.

For brevity experiments using isolated scales are referred to as in vitro. The results of the experiments involving light, heat and cold are described following Table 3.

4. Caudal bands

Caudal bands were produced by making a 2 mm cut in the caudal fin, using a fragment of razor blade. The Fundulus were anaesthetized with MS-222. After the cut was made the fish were placed in heavily aerated water to recover, and then put into numbered, clear plastic boxes. The anaesthetic was well tolerated and there were no deaths. The boxes were then placed in aquaria painted white inside and out, and given continuous illumination at an intensity of 150-foot candles by 150 Watt bulbs approximately 10 inches from the surface. The time for fading ranged from 10 hours to more than 14 days, and was characteristic for each fish.

The site of the cut was always apparent, so that caudal bands resulting from injury to the caudal fin during confinement could be distinguished easily. The fish were examined at least twice a day and the condition of the caudal band noted. Usually 24 hours or more were allowed after the band had faded before using the fish for experiment. This interval was necessary because the bands frequently showed what appeared to be spontaneous revival. Casual observations suggest that there may be circadian cycle of colour change, which may account for these apparent revivals.

After injection the fish were returned to the boxes in the illuminated white tanks and examined at 30-minute intervals for several hours, later at longer intervals.

For experiments involving fresh caudal bands the fish were injected as soon as they had recovered from the anaesthetic. They were then put into clear plastic boxes and placed on a white background in order to make the caudal band easier to see. Background exchanges were also carried out.

Records in both series of experiments consisted of noting the presence or absence of the band and the hour and date of the observation. A rough estimate of the degree of contrast of the band with the rest of the fin, and the overall tint of the body were also noted.

These methods produced some of the conditions of both the *in vivo* and *in vitro* experiments, and the results are shown in Table 4.

5. The influence of Ca^{++} on melanin movement

The method followed that suggested by Nash *et al* (1966). A scale was removed and placed in 0.2M EDTA diluted 1:50. The M.M.I. was calculated immediately and again after five minutes. The scale was then washed twice with 0.1M NaCl and returned to the EDTA solution. This procedure was repeated twice and the scale finally placed in 0.1M NaCl. The M.M.I. was calculated at five-minute intervals, usually for 15 minutes. The drug under test was then added and the M.M.I. recorded at five-minute intervals. If two drugs were to be tested, the first was removed with two washings with 0.1M NaCl, the M.M.I. in 0.1M NaCl recorded, and the second drug applied. Records were made as before. The duration of the EDTA bath and its concentration were also varied in these experiments. The time in the EDTA solution was increased up to a total of 45 minutes, and the concentration to 0.2M.

No controls were used, but reference was made to the numerous control records made in other *in vitro* experiments in analyzing the results of the experiments with EDTA. The results are described following Table 3.

6. Estimation of effects and methods of recording

It will be noted that although, as indicated above, experimental records in the *in vivo* and *in vitro* experiments were made in terms of the tinted papers and the M.M.I., which are semi-quantiative measures, the results are shown in the Tables in qualitative terms.

The problem of devising a satisfactory means of measuring and recording colour change has been a matter of concern for all who have studied this phenomenon. Wholly qualitative descriptions have only limited use, and the risk of subjective errors seems high. In an attempt to overcome this risk Hogben and Slome (1931) set up the Melanophore Index (M.I.), which assigns a numerical value from 1 to 5 to the degree of dispersion of melanin from the fully aggregated to the fully dispersed state.

Although the M.I. was clearly an advance, objections to it were raised, and alternative methods proposed (Hill, 1935; Smith, 1936; Parker, 1943; Thing, 1952; Wright, 1954; Deutsch *et al*, 1956; Teague, 1960; Long, 1961; Roggen, 1962). The newer methods employed transmittance or reflectance measurements. These methods, while eliminating the subjective element and capable of yielding apparently quantitative data, have important sources of error inherent in them. For example, reflectance methods measure only the amount of light reflected. In animals possessing other chromatophores in addition to melanophores, such measurements will almost certainly be erroneous. Transmittance methods are particularly useful in studying single melanophores. When these methods are used it is assumed that all other melanophores are acting in the same way as the cell under observation. In my experience this is by no means sure.

The papers by Thing and by Roggen, cited above, provide excellent discussions of the problem.

Healey (1940) was apparently the first to use the Ostwald papers as objective standards for comparing the tints of fish. His scale, slightly modified by deletion of the extremes and interpolation of a decimal fraction between the numbers, was used in making the experimental records set out in the present work.

In accordance with the conclusion reached by Thing (1952) that the M.I. was preferable to other methods of measuring colour change, it was decided to use the Index in an effort to eliminate subjective errors as far as possible without attempting to assign a quantitative aspect to the records. A change of one unit or more in the M.M.I. was arbitrarily designated as significant. A sustained change of 0.5 unit was considered indicative, and further tests were carried out in such instances.

To make such numerical changes meaningful in the Tables, it would be necessary to state the M.M.I. before and after each experiment. As these were almost never the same in each experiment, it will be seen that the incorporation of M.M.I. readings in the Tables would render them so bulky as to be virtually useless. It is hoped therefore, if the basis for the qualitative terms used in the Tables is kept in mind, no misunderstanding will arise.

The same considerations that apply to the M.M.I. apply to the use of the Derived Ostwald Index (Healey and Ross, in press) and the presentation of results derived from this method in qualitative terms in the Tables.

III. RESULTS

1. Injection experiments on intact fish

The results are presented in the form of three tables which summarize the data of many experiments (Tables 1, 3, 4).

The drugs applied in the three types of experiment, *in vivo*, *in vitro* and on caudal bands, are arranged according to the commonly accepted descriptions of the major effects in mammals for which they are best known, as briefly mentioned in the introduction. It is not proposed to describe in detail the characteristic effects of these drugs on mammalian tissues except where some special explanations for using them seem to be required. In general drugs were classified according to the descriptions in Goodman and Gilman (1965). However, in the discussion, references to the actions of these drugs in mammals will frequently be made when problems of interpretation arise. Appendix 1 lists the drugs used, giving chemical names, synonyms and names of manufacturers in order to provide an exact and complete record of the substances actually used in the tests.

Appendix 2 provides samples of the raw data from which the summary Tables 1-4 were compiled. These show the effects of carbachol *in vivo* and *in vitro*. This drug is important in the argument and the records were chosen to document its lack of effect under the experimental conditions.

Table 1 summarizes the major series of experiments. About 40 substances were tested, falling into eight groups with fairly clearly-defined properties along with about 10 agents which belong to no definite

group as regards their action on neuro-effector systems.

In an initial series of experiments an arbitrary dose of 100 mcg. was given. It was found that as a rule this dose was sufficient to show an effect, but if not, larger doses were given. If the initial dose gave rise to signs of distress or death, lower doses were given until a dose was found which would give reliable results. In the case of certain drugs of particular interest, e.g. adrenaline, noradrenaline, "Hydergine", etc., this procedure was continued until a minimum effective dose (m.e.d.) was determined. When it was noted that bretylium gave different results at high and low doses, other drugs with similar action were also tested at various dose levels. None had opposite effects under those conditions. No attempt was made to record the rate of onset of the effect. Where gross differences in time of onset or persistence of effect were observed, these are noted.

Attempts were made to determine whether the agent under investigation interfered with background adaptation. The results of these tests are shown in Table 1, under the heading Adaptation. The symbol "n.t." in this column indicates that the test was not carried out. As mentioned previously, Fundulus were adapted in pairs to white and black backgrounds. The drug was then injected and the effect recorded by comparing the fish with the grey-tinted papers of the Ostwald type. The backgrounds were then exchanged by transferring the fish in clear plastic boxes. Injected fish from the black background (BB) were thus observed on a white background (WB) to determine interference with adaptation to a white background (WA) and the other pair for interference with adaptation to a black background (BA).

In the Table no attempt has been made to give a quantitative assessment of the results; these are described simply as "A" (aggregating),

"D" (dispersing) or "O" (nil effect). However, the dosage provided does enable some quantitative comparisons to be made between different substances. Thus both adrenaline and serotonin have aggregating effects on intact Fundulus and are recorded as "A" but the effective dose required with serotonin is 50 times as strong. By comparing effective doses in this way one can see that there is a considerable difference in the effectiveness of the agents listed in the Table as aggregating or dispersing. This feature of the results must be taken into account in assessing significance later on.

When adrenaline and noradrenaline were injected after a number of blocking agents aggregation occurred in every case. This means that at the doses employed these drugs were unable to prevent adrenaline and noradrenaline from exerting their usual effect on the melanophores. These results do not indicate the same relationships that have been described between adrenaline and noradrenaline and these blocking agents which act at the nerve ending or on the effector. However, as might be expected, the ganglion blockers, hexamethonium and presidal did not oppose the aggregating effect of injected adrenaline, noradrenaline or ephedrine.

The timing and the doses of drugs used in pairs are critical. A detailed series of tests over a wider range of doses and with varying intervals between the two injections was beyond the scope of this investigation with the number of fish available. Ross and Healey (in press) working with the minnow Phoxinus also found that "Hydergine" and bretylium did not block the action of injected adrenaline, though they did obtain such a blockade with yohimbine and dibenzyline which were not used here.

Reserpine is frequently described as a depleter of catechol amines largely because in mammals they disappear from such sites as the adrenal medulla and sympathetic nerve endings when reserpine is present. The slow and prolonged dispersion with repeated doses of reserpine and the inhibition of adaptation to a white background is recorded in Table 1. It is possible that the catechol amines removed from their storage sites by reserpine are destroyed by monoamine oxidase, therefore the drugs parnate and pargyline were injected before reserpine to prevent the possible destruction of these catechol amines. Then reserpine was injected to see whether it still had its usual dispersing effect. With pargyline, (but not with parnate) aggregation occurred, when this MAO inhibitor was introduced 24 hours before reserpine. This suggests that under these conditions the catechol amines removed from the stores are still available to exert their normal effect.

A brief glance at Table 1 enables the following statements to be made: (1) most of the substances which show aggregating effects at low or moderate doses are catechol amines or allied substances; (2) substances with dispersing action at low or moderate doses are agents which block transmission in the sympathetic system or in sympathetic ganglia; (3) there is a virtual absence of significant effect at low or moderate doses of acetylcholine and any other substances which in mammals mimic the response to parasympathetic stimulation.

TABLE 1

Effects on Intact Fish of Agents Given by Intra-peritoneal Injection

Note: See end of Table for Legend of Abbreviations.

Agent	Dose	in vivo	Adaptation	Remarks
1. Sympathetic stimulating agents				
Adrenaline	m.e.d. 10 mcg	A	0	
Noradrenaline	m.e.d. 10 mcg	A	0	
Isoprenaline	100 mcg	A	n.t.	
Dopamine	m.e.d. 10 mcg	A	n.t.	
Tyramine	100 mcg	A	n.t.	
Ephedrine	100 mcg	A	n.t.	
Amphetamine	100 mcg	A	n.t.	
Tryptamine	m.e.d. 10 mcg	A	n.t.	
2. Sympathetic blocking agents -α-blockers				
Dibenamine	500 mcg	D	0	
Dibenzyline	100 mcg	0	0	
Tolazoline	m.e.d. 200 mcg	D	inhibits WA	
Regitin	100 mcg	D	n.t.	
Hydrogenated ergot derivatives ("Hydergine")	60 mcg	D	n.t.	

Table 1 continued

Agent	Dose	in vivo	Adaptation	Results
Sympathetic blocking agents -α-blockers cont.				
Yohimbine	100 mcg	D	n.t.	
Sympathetic blocking agents -β-blocker				
Propanalol	100 mcg	O	O	
3. Postganglionic sympathetic fibre blocking agents				
Bretylium	100 mcg	D	n.t.	
	10 mcg	A	n.t.	
Guanethidine	500 mcg	A	inhibits WA and BA	
4. Cholinergic (parasympathetic) stimulating agents				
Acetylcholine	10 mcg to 500 mcg	O	n.t.	No effective non-lethal dose found
Carbachol	O$\langle$10 mcg$\rangle$lethal	O	O	
Mecholyl	100 mcg	O	n.t.	
Eserine	10 mcg to 500 mcg	O	n.t.	No effective non-lethal dose found
Eserine and Acetylcholine	10 mcg to 200 mcg 10 mcg to 500 mcg	O	n.t.	No effective combination found

Table 1 continued

Agent	Dose	*in vivo*	Adaptation	Results
5. Cholinergic blocking agent				
Atropine	300 mcg	D	inhibits WA	Large doses cause persistent D
6. Ganglion stimulating agents				
Nicotine	100 mcg	A	inhibits BA	A *in vivo* is transient
Acetylcholine - after Eserine and Atropine	200 mcg 100 mcg 300 mcg	D (WA) A (BA)	inhibits WA	
7. Ganglion blocking agents				
Hexamethonium	100 mcg	D	n.t.	
Pentolinium	m.e.d. 10 mcg	D	inhibits WA	
Presidal	m.e.d. 30 mcg	D	n.t.	Persistent D *in vivo*
8. Monoamine oxidase inhibitors (MAOI)				
Parnate	100 mcg	A	inhibits BA	
Pargyline	100 mcg	O	n.t.	
9. Miscellaneous				
Serotonin (5HT)	m.e.d. 500 mcg	A	n.t.	

Table 1 continued

Agent	Dose	in vivo	Adaptation	Results
Miscellaneous cont.				
5-OCH_3 Tryptamine	500 mcg	A	n.t.	
3-OH Tyramine	100 mcg	A	n.t.	
Histamine	100 mcg	O	n.t.	
Melatonin	100 mcg to 500 mcg	O	n.t.	
Reserpine	100 mcg repeated	D	inhibits WA	Very slow onset
Chlorpromazine	100 mcg	A	n.t.	
Posterior pituitary powder	100 mcg to 500 mcg	O	n.t.	
10. Blocking agents followed by adrenaline, etc.				
Adrenaline	50 mcg			
after - Tolazoline	200 mcg	A	inhibits BA	
- Propanalol	100 mcg	A	inhibits BA	
- Dibenamine	100 mcg to 500 mcg	A	n.t.	
- "Hydergine"	60 mcg	A	n.t.	
- Presidal	100 mcg	A	n.t.	Later D
- Bretylium	100 mcg	A	n.t.	Later D
Noradrenaline	100 mcg			
after - Bretylium	100 mcg	A	n.t.	Later D
- Hexamethonium	100 mcg	A	n.t.	

Table 1 continued

Agent	Dose	in vivo	Adaptation	Results
Blocking agents followed by adrenaline, etc. cont.				
Noradrenaline	100 mcg			
after - Presidal	100 mcg	A	n.t.	Later D
Ephedrine	100 mcg			
after - Bretylium	100 mcg	inc.	n.t.	Effect varies with relative dose
- Presidal	100 mcg	0	n.t.	Lethal
- Hexamethonium	100 mcg	A	n.t.	Slight effect
Reserpine	100 mcg			
after - Parnate	100 mcg	0	inhibits WA	Given 24 hours before reserpine
- Pargyline	100 mcg	A	0	Given 24 hours before reserpine. Very slight effect
- Pargyline	100 mcg	D	inhibits WA	Given 15 minutes before reserpine

Legend of Abbreviations

A = Aggregation
D = Dispersion
0 = Nil effect
WA = Adaptation to a white background
BA = Adaptation to a black background
m.e.d. = minimum effective dose
n.t. = not tested
inc. = inconclusive

2. Experiments on isolated scales

(a) Drugs - In the experiments whose results are summarized in Table 3, most of the agents used previously in the intact fish were tested on isolated scales in order to determine whether the observed effect on the whole fish might be due to local action on the melanophore. The Table summarizes data obtained from successive readings of the melanophore indices over periods of time as described earlier. An example of a single such experiment is given in Appendix 2. In this example, carbachol was found to have no effect.

The appearance of melanophores in isolated scales in saline, and the changes in the degree of dispersion of melanin with time merit a brief description. The scale is almost completely transparent, and the melanophores and lipophores are clearly visible. The growth rings appear as semicircular darker lines. When the melanin is fully dispersed (M.M.I. = 5.0) the melanophores appear as very finely branched cells with a small, clearly defined pigment-free area at or near the centre of the cell. This area is probably the nucleus. The intricate branching and the central clear area give an over-all effect under the microscope which appears, paradoxically, less dark than the over-all effect at a lower M.M.I. (4.0). However, by selecting 10 melanophores and scoring only these repeatedly to derive the M.M.I., the optical illusion could be overcome. When the melanin was fully aggregated, (M.M.I. = 1.0), the melanophores appeared as round dots. No central clear area was visible.

The M.M.I. immediately after the scale was removed and placed in saline varied from some degree of aggregation down to M.M.I. 1.8, to almost complete dispersion up to M.M.I. 4.8. Regardless of the initial M.M.I. a period of fluctuating readings lasting in some instances up to one hour followed. The fluctuations tended to terminate in a steady

M.M.I. in the range 2.5 to 3.1, although steady but higher M.M.I.'s were sometimes recorded. Only after a steady M.M.I. had been maintained for at least 10 minutes was it considered that the fluctuations had ceased and the drug under test could be applied without the danger of confusing its effects with fluctuations. The observations summarized in Table 3 are, therefore, records of the drug-induced changes of the M.M.I. from this steady, intermediate M.M.I.

If the scale was maintained in saline at room temperature for much longer periods, up to four hours or more, a very slow decline in the M.M.I. to 2.0 to 2.4 was usually observed. This change was so slow, and usually so slight, that it is considered that the effect of it on the observations made during the relatively short period when a drug was tested would be extremely small. The effect, if any, would probably be noticeable only if the drug were relatively ineffective or at a high dilution, and only if the observation period were prolonged excessively.

The sample records given in Appendix 2 do not, perhaps, make clear another interesting feature of the behaviour of melanophores in isolated scales. At any time when the scale was in saline it was possible to find melanophores at various points with M.I.s ranging from about 2 or less to about 4 or more. In this circumstance melanin movement in all the melanophores was not synchronous. A similar lack of synchronous activity was frequently observed when an ineffective drug, or an effective drug at high dilution was applied. In the latter case, however, all the melanophores showed a similar response if the observations were continued for a sufficient period.

Melanophores which had been damaged during removal, usually by pressure, always remained in a state of partial dispersion (M.I. 3 to 4)

regardless of treatment. Damaged melanophores could be detected during the first part of an experiment and care was taken to exclude them from the 10 melanophores selected for scoring.

In view of the observations noted above, it may be useful to list here some agents causing aggregation and dispersion *in vitro*, together with the maximum, minimum and average changes of the M.M.I. induced by them. All data are taken from experiments in which the agents were applied at a concentration of 1 mg per ml, except as noted for adrenaline.

TABLE 2

Changes of Mean Melanophore Index Caused by Various Agents

Aggregating agents	Change of mean melanophore index		
	Min.	Max.	Av.
Adrenaline	0.2	0.6	0.4
" 0.01 mg/ml	0.6	1.3	0.9
Noradrenaline	3.5	3.6	3.5
Tyramine	0.6	3.1	2.2
Dopamine	1.0	2.5	2.0
Tryptamine	0.4	0.7	0.5
Serotonin	0.0	3.6	1.6
Dispersing agents			
Dibenamine	0.0	3.0	2.0
Tolazoline	0.2	1.9	1.3
Regitin	0.4	3.2	1.5
Nicotine	1.2	2.2	1.7
Pentolinium	0.9	2.3	1.5
Parnate	1.5	1.5	1.5
5-OCH_3 Tryptamine	1.1	2.9	1.8
Chlorpromazine	0.8	0.9	0.8

TABLE 3

Effects of Agents *in vitro* (on isolated scales)

Note: See end of Table for Legend of Abbreviations

Agent	Concentration	*in vitro*	Remarks
1. Sympathetic stimulating agents			
Adrenaline	1 mg/ml	A	m.e.c. 10 mcg
Noradrenaline	1 mg/ml	A	m.e.c. 10 mcg Occasionally D *in vitro* at low conc.
Dopamine	1 mg/ml	A	m.e.c. 10 mcg
Tyramine	1 mg/ml	A	
Tryptamine	1 mg/ml	A	m.e.c. 10 mcg
2. Sympathetic blocking agents -∝-blockers			
Dibenamine	1 mg/ml	D	
Tolazoline	1 mg/ml	D	
Regitin	1 mg/ml	D	m.e.c. 10 mcg
Hydrogenated ergot derivatives ("Hydergine")	1 mg/ml	O	
3. Postganglionic sympathetic fibre blocking agent			
Bretylium	1 mg/ml	O	
	10 mcg/ml	O	

Table 3 continued

Agent	Concentration	in vitro	Remarks
4. Cholinergic (parasympathetic) stimulating agents			
Acetylcholine	1 mg/ml	O	
Carbachol	1 mg/ml	A(?)	
Eserine	1 mg/ml	O	
5. Cholinergic blocking agent			
Atropine	1 mg/ml	O	
6. Ganglion stimulating agent			
Nicotine	1 mg/ml	D	
7. Ganglion blocking agents			
Hexamethonium	1 mg/ml	O	
Pentolinium	1 mg/ml	D	
Presidal	1 mg/ml	O	
8. Monoamine oxidase inhibitor (MAOI)			
Parnate	1 mg/ml	D	
9. Miscellaneous			
Serotonin (5HT)	1 mg/ml	A	
5-OCH_3 Tryptamine	1 mg/ml	D	

Table 3 continued

Agent	Concentration	in vitro	Remarks
Miscellaneous cont.			
3-OH Tyramine	1 mg/ml	O	
Histamine	1 mg/ml	O	
Melatonin	1 mg/ml	D(?)	
Reserpine	1 mg/ml	O	
Chlorpromazine	1 mg/ml	D	
Posterior pituitary powder	1 mg/ml	O	

Legend of Abbreviations

A = Aggregation
D = Dispersion
O = Nil effect
m.e.c. = minimum effective concentration

(b) Chelating agent (EDTA) - The object of these experiments was to find out if the introduction of EDTA to remove Ca^{++} affected the aggregation or dispersion of melanophores in response to drugs. There is growing evidence that many if not all membrane phenomena are calcium dependent.

The results of experiments with EDTA *in vitro* may be described as follows: As a rule the melanin dispersed during the procedure to remove Ca^{++} and usually aggregated in the 0.1M NaCl bath used at the end of the procedure. Normally there was slow aggregation in saline. page 25, As was reported earlier, this is what usually happens when untreated scales are placed in saline. Then dibenamine was applied at a concentration of 1 mg per ml; it has been shown above that dibenamine causes dispersion under these conditions. After EDTA it also caused dispersion and this could be reversed by washing and leaving the scale with 0.1M NaCl. A parallel experiment was done with adrenaline. It was found that after EDTA, adrenaline still caused aggregation. This aggregation however, was not reversed in 0.1M NaCl.

Two further tests were made to determine whether the mechanisms for aggregation and for dispersion were still intact after EDTA treatment. Dibenamine was applied after EDTA, and dispersion occurred as before. The scale was then washed twice with 0.1M NaCl and placed in adrenaline (1 mg per ml). Aggregation was observed, although usually not to the same extent as that caused by adrenaline alone *in vitro*; in some experiments aggregation failed to appear. If aggregation did occur, the scale was washed as before and dibenamine again applied. Dispersion followed, but more slowly than before.

These results indicate that exposure to EDTA even at relatively high concentrations does not prevent the melanophore from exhibiting both

aggregation and dispersion of melanin, and therefore, that neither is a calcium-dependent process. This conclusion must be regarded with some caution. Although dispersion during the EDTA treatment, and aggregation in 0.1M NaCl following the treatment were constant features, the effects of drugs applied subsequently were not always reproducible. The possibilities that, because of slow diffusion, the chelating agent did not reach all the calcium in the scale, or that Ca^{++} was inadvertently introduced as a contaminant of the reagents, cannot be overlooked.

(c) Physical stimuli - The preparation used in in vitro experiments suggests by its very nature a means of determining whether the melanophore can act as an independent effector and if so, its responses to natural physical stimuli. As variations in illumination and temperature may occur in the course of an in vitro experiment it was decided to test the effects of continuous illumination, cold, and heat on melanophores which might be expected to respond as independent effectors.

The evidence supporting the concept of the melanophore as an independent effector is that many chromatic animals, including Fundulus, still respond to changes in illumination after blinding. The response to the background is, of course, abolished, but bright illumination causes dispersion and dim light or darkness causes aggregation. These findings and their possible interpretation are discussed in Parker (1948) and Waring (1963). Waring and Landgrebe (1950) provide strong arguments that the melanophore may under certain conditions act as an independent effector.

The procedure carried out to determine the effect of the physical stimuli light, heat and cold on the melanophores in an isolated scale from Fundulus has been described previously, page 10. Light had no effect, the

M.M.I. very gradually decreasing over periods of a least one hour after the customary initial slight fluctuations. At the temperature of melting ice (about 0° C) the melanophores showed aggregation. This aggregation could be reversed by allowing the saline in which the scale was placed to warm to room temperature (23°-26° C) or by replacing the cold saline with saline at room temperature. The M.M.I. usually returned to a level above that recorded before cooling, although occasionally the M.M.I. would be somewhat below this level. In the experiments involving cooling and warming, the scale was illuminated only long enough to read the M.M.I. Warming from room temperature to 32.5° C also caused aggregation in the melanophores. This could be reversed by allowing the saline to cool to room temperature or by replacing the warm saline with saline at room temperature. The M.M.I. then rose to a level somewhat below that recorded before warming.

The results of the experiments in which light was the only physical stimulus acting indicates that the melanophore does not respond to light as an independent effector. This is in contrast to the effect of light on blinded animals, and suggests that a non-visual receptor other than the melanophore is involved.

The results obtained by applying temperature extremes are more difficult to interpret. The point is often raised in discussion of in vitro experiments that the isolated scale is not really denervated because the torn terminations of the nerve fibres still adhere to the scale. The responses of melanophores in vitro may therefore be influenced by injury currents in the amputated fibres, and the effects of various agents may be due to their action on the torn ends of the fibres rather than on the melanophore directly. With this possibility in mind, the results may be explained on the basis of a single, aggregating, nerve

fibre responding to thermal stimuli. If two opposing nerves with different thresholds to various stimuli were involved in melanophore control, as Parker held, it might be expected that the temperature extremes used in these experiments would have different effects rather than the single effect of aggregation observed.

If however the melanophores of isolated scales are regarded as denervated, then it may be said that they can respond to thermal stimuli as independent effectors. The question might be resolved definitely by testing the response of chronically denervated melanophores to heat and cold.

A review of the results of experiments summarized in Table 3 will bring out the following general observations: (1) as with intact fish aggregation occurs only with catechol amines or allied substances, except for serotonin; (2) there is no evidence of consistent aggregation or dispersion by acetylcholine and associated drugs, e.g. those which potentiate (eserine), or those which block (atropine) the action of acetylcholine in mammals; (3) some but not all the substances which caused dispersion in the intact fish caused dispersion in the isolated scales; this feature of the results needs some amplification. A basic difficulty in comparing the results with intact fish and isolated scales is that the concentrations of the agents introduced at the melanophore itself cannot be exactly determined. Drugs were tested on isolated melanophores over a range of concentrations and those shown in Table 3 are maximal. They are certainly much higher than any concentrations that could be achieved by the doses injected. It follows that substances which have effects on intact Fundulus and not on the isolated melanophores ("Hydergine", bretylium, hexamethonium) cannot be acting directly on the melanophores but must be affecting some other element in the mechanism of colour

change. This is understandable with hexamethonium, a ganglion blocker; it conforms with the general view that bretylium blocks the release of transmitter; it suggests that "Hydergine" must be acting on the transmitting step and not on the processes in the melanophore itself.

Because of the difficulty of equating doses in intact fish and on isolated melanophores, discussed above, it cannot be concluded that substances like dibenamine, regitin, etc., having dispersing effects on isolated scales owe their dispersing effects in intact fish to their direct action on melanophores. Only regitin was tested at lower doses; as shown in Table 3 it still caused dispersion in isolated scales when diluted 100 times below the standard concentration used in these experiments. In this case a direct action on the melanophores might be suggested to explain the dispersing action in the whole fish. Further work with lower doses will be needed to classify the action of the other substances of this kind.

3. Injection experiments on fish with caudal bands

Table 4 shows the effects of a number of the same substances listed in Table 1, in animals with caudal bands prepared as previously described. In this set of experiments it was expected that the effects of aggregating agents only would be evident in fresh caudal bands, because the melanophores in such bands are in a dispersed state.

In testing the effects of drugs on fresh caudal bands the experiments were acute, in the sense that the drugs were administered as soon as possible after the cut in the caudal fin was made. Faded caudal bands were formed by maintaining the Fundulus on an illuminated white background until the band had not been visible for at least 24 hours. The agent under test was then injected.

The letter symbols used in Table 4 have a somewhat different meaning than in Table 1. In order that there may be no confusion about the meaning of the results of these experiments, let us examine a single example in Table 4--say hexamethonium. In the animals with freshly prepared caudal bands, the Fundulus on the white background was pale except for the caudal band. Injecting hexamethonium into this fish caused dispersion in the whole fish compared to which the caudal band appeared paler, and hence the symbol "A" is used to describe the effect in Table 4. It must be stressed therefore that in this context the symbol "A" does not necessarily mean that aggregation has occurred in the melanophores of the caudal band, but that compared with the rest of the fish the band appeared paler. The injection of hexamethonium into fish with faded caudal bands (done several days after the cut was made) resulted in dispersion in the whole fish, but the effect was more marked in the caudal band. In the other situation, with Fundulus with a faded caudal band on a black background, injecting hexamethonium eliminated

the contrast between the faded caudal band and the rest of the fish, hence the symbol "O".

One interesting feature of the results was that most of the drugs which were effective in the intact fish had similar effects on the caudal bands as on the rest of the fish. This indicates that the circulation to the caudal bands had not been impaired by the denervating operation.

The fresh caudal bands stand out as dark stripes on fishes on a white background. When an aggregating agent is injected the band pales, and in the case of tyramine, the band becomes paler than the rest of the body. There is no obvious explanation why the melanophores in the freshly denervated band should be more sensitive to tyramine than those in the rest of the body. Tyramine is best known as a releaser of noradrenaline (Burn and Rand 1960) but this does not seem to have any relevance to the observation reported here.

The adrenergic blocking agents, with the exception of bretylium, have the same effects on the fresh caudal bands as on the rest of the fish. With bretylium, the band remains darker when Fundulus is on a white background and pales when it is on a black background. This suggests at least that the site of action of bretylium is not on the melanophore itself nor at the neuro-effector junction. The effect of hexamethonium on the fresh caudal band is also in keeping with its effect in mammals. It would be expected that this ganglion blocker would not show an effect in a denervated area and the band and the rest of the body would show a contrast.

It will be recalled that faded caudal bands are produced by maintaining a Fundulus with a cut in the caudal fin on an illuminated white background until the dark stripe fades to match the rest of the body. None of the sympathetic stimulating agents affect the caudal

band differently to the rest of the body, and both body and band are equally pale on either background.

The sympathetic blocking agents and post ganglionic fibre blocking agents mostly cause the band to appear darker than the rest of the body, both on white and black backgrounds. Before attempting to interpret this one needs to ask the question why the caudal band fades after a period of time. One possibility would be based on the assumption that in fishes, like higher vertebrates, there are circulating catechol amines from the suprarenal glands. It would be reasonable to expect the denervated melanophores to become hypersensitive to these substances so that after a time the melanin becomes more aggregated. Under such circumstances on the denervated melanophores the blocking agent will counteract only the aggregating influence of circulating catechol amines. Elsewhere it will also have to counteract the influence of the transmitter which the nerve ending would be delivering in close proximity to the melanophore, and so its dispersing effect would be less marked. A general significance to be attached to the caudal band experiments will be discussed below.

TABLE 4

(See text for special meanings of the symbols "A", "D", "O" in these experiments.)

Effects on Caudal Bands of Agents Given by Intra-peritoneal Injection

Note: See end of Table for Legend of Abbreviations

Agent	Dose	Caudal bands: Fresh WB	Fresh BB	Faded WB	Faded BB	Remarks
1. Sympathetic stimulating agents						
Adrenaline	m.e.d. 10 mcg	O	O	O	O	
Noradrenaline	m.e.d. 10 mcg	O	O	O	O	
Isoprenaline	100 mcg	O	O	nt.	nt.	
Tyramine	100 mcg	A	A	O	O	Faded CB becomes red
Ephedrine	100 mcg	O	O	O	O	
Amphetamine	100 mcg	D	A	O	O	
Tryptamine	m.e.d. 10 mcg	nt.	nt.	O	O	
2. Sympathetic blocking agents -∝-blockers						
Dibenamine	500 mcg	nt.	nt.	D	D	Slow revival of faded CB
Dibenzyline	100 mcg	O	O	O	O	
Regitin	100 mcg	nt.	nt.	D	D	

Table 4 continued

Agent	Dose	Caudal bands: Fresh WB	Caudal bands: Fresh BB	Caudal bands: Faded WB	Caudal bands: Faded BB	Remarks
Sympathetic blocking agents -α-blockers cont.						
Hydrogenated ergot derivatives ("Hydergine")	60 mcg	nt.	nt.	O	A	Retards D of faded CB on BB
Yohimbine	100 mcg	O	O	D	D	
Sympathetic blocking agents -β-blocker						
Propanalol	100 mcg	nt.	nt.	O	O	Retards D of faded CB on BB
3. Postganglionic sympathetic fibre blocking agents						
Bretylium	100 mcg	D	A	D	D	
Guanethidine	500 mcg	O	O	D	A	
4. Cholinergic (parasympathetic) stimulating agents						
Acetylcholine	10 mcg to 500 mcg	No effective non-lethal dose found				
Carbachol	O<10 mcg>lethal	No effective non-lethal dose found				
Eserine	10 mcg to 500 mcg	No effective non-lethal dose found				
Eserine and Acetylcholine	10 mcg to 200 mcg 10 mcg to 500 mcg	No effective non-lethal dose found				

Table 4 continued

Agent	Dose	Caudal bands: Fresh WB	Caudal bands: Fresh BB	Caudal bands: Faded WB	Caudal bands: Faded BB	Remarks
5. Cholinergic blocking agent						
Atropine	300 mcg	nt.	nt.	D	A	Very slow action on CB
6. Ganglion blocking agents						
Hexamethonium	100 mcg	A	A	D	O	
Presidal	m.e.d. 30 mcg	O	O	D	D	
7. Monoamine oxidase inhibitors (MAOI)						
Parnate	100 mcg	nt.	nt.	O	A	
Pargyline	100 mcg	nt.	nt.	O	O	
8. Miscellaneous						
Serotonin (5HT)	500 mcg	O	O	O	O	
5-OCH_3 Tryptamine	500 mcg	nt.	nt.	O	D	
3-OH Tyramine	100 mcg	O	A	O	O	
Histamine	100 mcg	nt.	nt.	O	O	
Melatonin	100 mcg to 500 mcg	nt.	nt.	O	O	
Reserpine	100 mcg	O	O	nt.	nt.	
Chlorpromazine	100 mcg	O	O	O	O	

Table 4 continued

Agent	Dose	Caudal bands: Fresh WB	Caudal bands: Fresh BB	Caudal bands: Faded WB	Caudal bands: Faded BB	Remarks
Miscellaneous						
Posterior pituitary powder	100 mcg to 500 mcg	nt.	nt.	O	A	

Legend of Abbreviations

A = Aggregation
D = Dispersion
O = No contrast
m.e.d. = minimum effective dose
CB = Caudal band
WB = White background
BB = Black background
nt. = not tested

IV DISCUSSION

1. The mechanism of nervous control

In animals possessing melanophores under nervous control, the presence of aggregating nerve fibres seems to be universally admitted (Parker 1948, Waring 1963, Fingerman 1965). It is also generally conceded that these fibres are a part of the sympathetic division of the autonomic nervous system. The results reported here are in agreement with this concept. All sympathetic stimulating agents caused aggregation *in vivo* and *in vitro*. Adrenaline and noradrenaline are the chemical transmitters in adrenergic systems (von Euler 1951). There is evidence that isoprenaline acts principally on the β-receptors of mammalian vascular smooth muscle, causing vasodilation, while in similar preparations adrenaline and noradrenaline act principally on the α-receptors causing vasoconstriction (Ginsburg and Cobbold 1960). No corresponding differential effect of these substances on the melanophores was noted. Other agents in this group may owe their effect to the fact that they are precursors of the transmitters, for example dopamine, or that they have been shown to cause the release of transmitters in other animals, for example tyramine (Burn and Rand, 1958 and 1960; Burn 1963).

Further support of the concept of an adrenergic mechanism for the aggregation of melanin in *Fundulus heteroclitus* is provided by the effects of adrenergic blocking agents *in vivo* and *in vitro*. The α-adrenergic blocking agents caused dispersion in both instances, suggesting that in this fish these agents act at the neuro-effector junction. The β-adrenergic

blocker, propanalol, on the other hand, had no effect. The evidence presented here is admittedly scanty, yet it seems to suggest the possibility that only α-receptor sites on the melanophore are involved in pigment aggregation.

The evidence from these experiments for the presence of a cholinergic dispersing mechanism is entirely negative. This is in contradiction to Parker's (1934a) conclusion. He injected eserine (0.2 cc of a 1:10,000 solution) and found no effect on black or white adapted Fundulus. Twenty minutes later he injected acetylcholine (0.2 cc of a 1:10,000 solution) and reported that the black-adapted fish paled while the white-adapted fish showed no colour change. Somewhat incomprehensibly, Parker concluded the acetylcholine caused dispersion. He noted that acetylcholine had no effect on caudal bands and that the concentration used was frequently lethal. The results presented here are in agreement with these latter observations. Parker (1943a) carried out similar experiments on catfish and obtained dispersion, and later Parker (1948) generalized these and the results reported by others in a statement that acetylcholine causes dispersion.

Repeated attempts were made to demonstrate an effect by acetylcholine on Fundulus melanophores in vivo and in vitro, without success. Doses of both acetylcholine and eserine were given at many concentrations. It is true that very high concentrations of acetylcholine in vivo, or somewhat lower concentrations after eserine caused darkening. Little significance can be attached to this observation, as such darkening was always associated with other signs of extreme distress and was usually followed by death. Darkening associated with signs of distress and approaching death was a common observation after administration of toxic or lethal doses of drugs, or when the fish became moribund from other causes.

In passing it may be noted that death in a blanched state may be brought about by lethal doses of adrenaline, noradrenaline and some other agents causing aggregation at sub-lethal doses. In these cases, as in the case of acetylcholine, it is assumed that the colour change is secondary to the toxic effects elsewhere. Synthetic cholinergic agents, which might be expected to be less readily inactivated, were also ineffective or lethal.

From these results it may be concluded that acetylcholine does not function as a mediator involved in dispersion at the neuro-effector junction in the chromatic system in Fundulus. The slow dispersing action of atropine in vivo and in vitro does not seriously contradict this conclusion, because it should cause aggregation if dispersion were mediated by acetylcholine. Perhaps mention should be made of a possible alternative explanation, involving presumed postganglionic cholinergic sympathetic fibres (Burn and Rand, 1960; Boyd et al, 1960). These authors used mammalian material and they advanced the above explanation to account for responses to acetylcholine after adrenergic blockade. The idea is suggestive, but as the effects of acetylcholine after adrenergic blockade were not tested in Fundulus it would be unprofitable to discuss it here.

Atropine has both central and peripheral effects in mammals (Goodman and Gilman 1965). The central effects at high doses are principally excitation which may be followed by depression and death. The peripheral effects of atropine are linked to apparent prevention of the combination of acetylcholine with cholinergic receptors on the effector cell membrane. It is probable that the slow persistent darkening following very large doses (Table 1) was due more to the central effects than to peripheral action (Table 3). Furthermore, Bainbridge and Brown (1960) point out that atropine is about as potent

a ganglion blocker in mammals as hexamethonium, although less persistent. On Fundulus the two drugs had similar effects in vivo (dispersion) and no effect in vitro, suggesting that the site of action of atropine was, like hexamethonium, at the ganglion.

In connection with the effect of atropine on the chromatic system, Fänge's (1962) review should be consulted. Papers by Gilson (1926), Smith (1931), Kamada and Kinosita (1944), and Fujii (1960) support the concept that atropine has little or no effect on fish melanophores in vivo and in vitro. Contrary evidence is given by Pouchet (1871c), Spaeth (1916a), Wyman (1924a), Matthews (1931), Wilber (1954, 1960), Kinosita (1963) and Pye (1964). These latter workers obtained dispersion with atropine.

These contradictions concerning the effects of atropine applied in vivo and in vitro that have been recorded in the literature and in these experiments on Fundulus make it difficult to use these results for the interpretation of the mechanism of nervous control of the melanophores.

Additional though somewhat indirect evidence for the presence of an adrenergic neuro-effector mechanism may be drawn from the in vivo dispersing effects of ganglion blocking agents, and postganglionic fibre blocking agents (e.g. hexamethonium, bretylium). Also, indirect evidence for the absence of an antagonistic cholinergic mechanism may be drawn from the aggregating effect of nicotine in vivo. In the discussion, the result of attempts to demonstrate the nicotinic action of acetylcholine will be ignored. The fish showed many signs of extreme distress in these attempts and for this reason little significance can be attached to the result.

As pointed out by Goodman and Gilman (1965) the effects of ganglionic blockade can be predicted if one knows whether the organ is receiving fibres from the parasympathetic or the sympathetic division of the

autonomic nervous system. It seems reasonable to assume that the same analysis applies to ganglionic stimulation. Applying this argument to the observations reported here that sympathetic stimulating agents caused aggregation *in vivo* and *in vitro*, one may conclude that the melanophores are innervated solely by the sympathetic nervous system. Similarly, since parasympathetic stimulating agents were ineffective in bringing about colour change it may be argued that there is no parasympathetic innervation of the melanophore.

Agents known to cause ganglion blockade and stimulation resulted in dispersion and aggregation respectively. If the argument outlined above is correct, these results strongly suggest that the postganglionic fibres are predominantly, if not wholly, adrenergic. Wilber (1954) also obtained dispersion in *Fundulus* by injecting hexamethonium (at the very high dose of 5.0 mg), but ascribed the effect to depression of the central nervous system. It is significant that nicotine had opposite effects on the melanophore *in vivo* and *in vitro* (Tables 1, 3), and that the *in vivo* effect was opposite to that of the ganglion blocking agents.

The effects of all agents tested, which block the autonomic nervous system at various levels, are consistent with the suggestion that the postganglionic fibres innervating the melanophores are adrenergic. Some act on the postsynaptic membrane (e.g. hexamethonium), others seem to impair conduction in the sympathetic neurone (e.g. bretylium). The majority, which were adrenergic blocking agents, appear to act on receptors on the effector. The sites and modes of action of blocking drugs are discussed by Nickerson (1949), Paton and Zaimis (1952), Toman (1952), Boura and Green (1959), Boura *et al* (1960) and Goodman and Gilman (1965).

The paradoxical aggregation following small doses of bretylium and much larger doses of guanethidine may be explained on the basis of their known action of releasing catechol amines from nerve endings.

(Goodman and Gilman 1965; Burn 1963; Boura and Green 1959). However, Bein (1960) expressed doubts concerning the releasing action of guanethidine.

It is recognized that the evidence and discussion presented here concerning the function of postganglionic fibres is such that firm conclusions cannot be drawn. As the diverse actions of drugs affecting these structures become more fully understood, further study of their effects on vertebrates with pigmentary effectors would be profitable. In any case, the warning by Nickerson (1959) that "all available agents have important actions in addition to blockade of responses to adrenaline and noradrenaline" (p. 448) should be kept in mind.

To sum up, evidence for an aggregating adrenergic mechanism in Fundulus seems ample and conclusive. Evidence for a cholinergic dispersing mechanism, based on the drug effects, is totally unconvincing.

A clear understanding of the active and resting state of the melanophore, as expressed by the degree of pigment dispersion, is basic to a full understanding of drug effects upon the cell. Here again opinion seems to be divided between (1) those who regard the dispersed condition as a passive non-aggregated state and (2) those who regard dispersion as an active state opposing aggregation in the way that excitation and inhibition are so often opposed in autonomic effectors in higher vertebrates. These two propositions merit discussion.

The former of these two groups argue basically by analogy with autonomic systems generally. Electrical stimulation of the sympathetic nervous system and drugs known as sympathetic stimulating agents from their mammalian effects cause aggregation of the melanin in the melanophores. Dispersion is not caused by electrical stimulation under any normal circumstances nor by drugs known as parasympathetic stimulating

agents. On this basis Spaeth (1913 and 1916a, b), Wyman (1924a), Osborn (1938), Falk and Rhodin (1957), and Dikstein and Sulman (1964) have all adopted a mononeuronic hypothesis of nervous control of melanophores, regarding the aggregated condition as the active state and the dispersed condition as a resting state. For higher vertebrates, Burn (1950) has pointed out that for some effectors the same chemical mediator may give rise to opposing actions, depending upon the concentration of the mediator. A high concentration of acetylcholine for example, may have an inhibitory effect while a lower concentration has an excitatory effect.

Yet there are some reasons for thinking that the dispersed state is not a passive non-aggregated condition. One problem that supporters of the mononeuronic hypothesis find difficult to explain is that the melanophores do not go to full dispersion in total darkness. Full dispersion is only obtained when the animal is exposed to an illuminated black background, suggesting a second, active, process of some kind.

Parker was the leading exponent of the view that dispersion is an active process. One of his arguments was based on an experiment in which a cold block was placed in the path of a caudal band and this blocked the spread of the dispersion that normally occurs in such bands (Parker 1935b). He argued that if dispersion were a passive process, it would not be blocked in this way. According to Parker's definition the active state is associated with movements of melanin granules, and the resting state by a lack of movement. This implies two kinds of movement under two kinds of nervous control. This definition seems to have been accepted by Waring (1963). Other definitions involving excitation and inhibition by similar or different agents are hypothetical and have no experimental basis in work on Fundulus.

The observations of Lerner and Takahashi (1956) and Lerner (1959), using frogs, that oxygen was essential for the maintenance of dispersed melanin, but not for aggregated melanin, cast some doubt on the validity of comparing the active state of the melanophore to that of other cells. It seems clear that since dispersion requires oxygen it is probably an active process and not merely the result of cessation of activity in the aggregating fibres. But it would be a mistake to assume that teleost fish also require oxygen to maintain dispersion without direct evidence. It would be profitable to apply Lerner's approach to fish melanophores. In some ways the dispute concerning the active and resting states of the melanophore is reminiscent of the long standing argument as to whether relaxation of muscle is an active or a passive process. Nevertheless it is important to recall that only Parker was able to produce dispersion in response to electrical stimulation of Fundulus, and only under rather special circumstances.

A continuing source of confusion in the investigation of chromatophore physiology arises from the use of different terminologies by different authors. Parker (1948), Jenkin (1962) and Waring (1963) have discussed these terminological problems extensively. A particular source of difficulty is the term "neurohumor" introduced by Parker (1934b) to describe the substances liberated at the terminals of chromatic nerves and causing changes in the state of the chromatophore. In 1940 Parker enunciated a more complete definition: "A neurohumor is a hormone produced by nervous tissue or glands appended to nervous tissue serving as an activator or inhibitor of other nervous tissue or its effectors". The term includes acetylcholine and intermedin, but excludes secretin, androgens and estrogens. He noted that neurohumors act only in the nervous system, and that they are also transmitters at the synapse and

neuro-effector and neuro-receptor junctions. This function was distinguished from another form of control designated "pure nerve action", while admitting that neurohumors were equivalent to chemical transmitters. It seems evident from this description that the equivalence of chemical transmitters and "pure nerve action" was not clear, probably because the general theory of chemical transmission of nervous activity was not well established at that time. The term neurohumor now gives rise to other difficulties in view of the widespread occurrence of neurosecretion and neurohormones, substances produced by nerve cells and conducted along nerve fibres. In this work the term chemical transmitter is used instead of neurohumor. However, because of Parker's great contributions to the study of chromatophore physiology, and especially to emphasize the equivalence referred to above, the term neurohumor will be inserted where such emphasis seems to be required. No distinction will be made between hormones, sensu stricto and neurohormones.

2. Some evolutionary implications of nervous control of pigment movement.

Poikilothermic chordates known to be capable of the background response may be grouped into three classes on the basis of the mechanism of control (Fingerman 1965). The first class includes all forms having hormonal control only; the second those having nervous control only; and the third, comprising those animals with both hormonal and nervous control.

The means of distinguishing the two types of control are convincing for the first two classes. Nerve section will have no effect on the colour responses of the denervated area in animals having only hormonal control. The hormone or hormones involved may be deduced from the effects of hypophysectomy and the injection of chromatophore-activating hormones

into animals with and without a hypophysis.

Nerve section in animals of the second class always has profound effects on the background response. Melanophores in the denervated area show no response to background, showing a static condition which may be opposite to that of the melanophores of innervated regions. In animals with purely nervous control, hypophysectomy and hormone injections will have no acute effect on melanophores.

A third method of distinguishing between hormonal and nervous control is based upon the time required for colour change (Neill, 1940). Briefly stated, if colour change is complete in 10 minutes or less, the control is nervous. If the time required is two hours or more, control is hormonal. Mixed hormonal and nervous control would therefore give total change times between 10 and 120 minutes, the actual time presumably indicating the relative predominance of the two means of control. Using this criterion of time, Neill had concluded that cyclostomes, elasmobranchs and amphibia had humoral control, and reptiles and some teleosts had nervous control.

Although Neill's criterion provides a useful rough guide, it has been criticized by Parker (1948) on the grounds that it was based on an insufficient sample. As more data on total change time accumulated it became apparent that chromatic vertebrates are on a continuum rather than in three discrete classes in regard to time for complete physiological colour change. Waring (1963) defended the criterion by elaborating on Neill's consideration of the three temporal components of total change time. The total change time is the sum of the delay at the receptor, before the stimulus acts upon the receptor, the time required for conduction of the action through the coordinating system, and the time required for completion of the response by the effector. Waring pointed

out that in nervous control the first two components will require a few minutes at most. If the total change time is much more than a few minutes the delay must be in the effector response. To make the criterion decisive it is necessary to determine the time required to complete the effector response to the suspected control agent: chemical transmitters in the case of suspected nervous control, and appropriate hormones in the case of suspected hormonal control.

In order to ensure that the melanophore is free from extraneous influences when determining the effector response time, the determination might well be carried out *in vitro*. In putting this to the test in *Fundulus*, one gets the anomalous result that the time required for the melanophores to respond to noradrenaline *in vitro* is greater in my experience than the time required for adaptation of the whole fish to a white background. The delay here probably corresponds to the time required for the agent to diffuse from the medium to the effector as compared to the delay at the neuro-effector junction *in vivo*. It may be concluded that until more precise data on the three temporal components of the total change time are available, Neill's (1940) criterion should be given only qualitative significance. Fishes, like *Fundulus*, which can change colour in less than five minutes at normal temperatures, are clearly not exclusively hormonal. Similarly, fishes that take many hours or days to change colour almost certainly have a hormonal component in their mechanism of control.

The results reported here offer no evidence of hormonal control of colour change in *Fundulus heteroclitus*. It is conceded that the experiments were not carried out on fish which had been blinded, hypophysectomized, or both. The results obtained by other workers on such preparations suggested that to attempt to carry out such experiments with the number

of fish available to me would have jeopardized the rest of the experimental program. It has been shown repeatedly that blinding abolishes physiological colour change in Fundulus and other teleosts (Pouchet, 1871a, 1874, 1876; von Frisch, 1911; Smith, 1928; Osborn, 1939; Parker, 1941). Such fish do not usually show pronounced aggregation or dispersion following the operation, but may still respond to changes in illumination (Parker 1948).

Apparently hypophysectomy does not abolish completely the background response in Fundulus (Matthews 1933, Abramowitz 1937, Pickford and Kosto 1957, Waring 1963). The operation has no effect on the background response in Phoxinus (Gray 1956), and prevents full dispersion in catfish (Osborn 1938). Healey (1940, 1948) found on the contrary that hypophysectomy interfered with the maintenance of background adaptation in Phoxinus.

The literature reveals that opinion as to the action of the pituitary and its importance in colour change in teleosts is by no means unanimous. Abramowitz (1937c) found in Fundulus that innervated melanophores were little affected by hypophysectomy, but that denervated melanophores could not disperse fully. He attributed the deficient response to lack of hypophyseal hormone in the blood, and was satisfied that there was sufficient melanophore hormone in the pituitary and in the blood to establish it as a significant agent in the melanophore response. The only effect of posterior pituitary powder in my experiments was a scarcely detectable slowing of the gradual darkening of a faded caudal band when the fish was over a black background.

Hogben and Winton (1916, 1922) had demonstrated that extracts of the pituitaries of fish, amphibians, reptiles and birds contained a melanin-dispersing agent. Frog melanophores were used to demonstrate the effect. Kent (1959) adapted Phoxinus to a white background, removed the pituitary and divided it into anterior and posterior halves.

Injection of homogenates of each half evoked aggregation in a black-adapted fish, more marked when the anterior half was injected. Matthews' (1933) observation that removal of the pituitary did not affect background adaptation in Fundulus has been noted. He found in addition that Fundulus pituitary extract caused aggregation in vitro. Kleinholz (1935) affirmed that the hypophysis of Fundulus heteroclitus contained a substance which would cause dispersion in denervated Fundulus melanophores in vivo and normal melanophores of the catfish, frog and lizard. Innervated Fundulus melanophores did not respond to Fundulus pigmentary hormone, nor to amphibian or mammalian pituitary extracts. The role of the pituitary in the colour change of catfish is apparently similar to that in Fundulus (Osborn, 1938). He found that catfish lacking a pituitary could not darken fully and that injection of a pituitary extract would cause dispersion. Pickford (1957 and 1959) investigated pituitary function in many fish, including Fundulus. She concluded that in Fundulus only denervated melanophores respond to intermedin. Her results coincided with Kent's (1959) finding that there is also an aggregating agent in the pituitary.

Finally, Palay (1953) studied the anatomical and histological effects of hypophysectomy on the brain of Fundulus. In the hypothalamus he found cells containing neurosecretory granules and sending axons to the neurohypophysis. After hypophysectomy the neurosecretory cells degenerate. He suggested that posterior pituitary hormones may be synthesized in the hypothalamus and stored in the neurohypophysis.

The confusing and sometimes contradictory evidence cited above may reflect either a wide range of function of the hypophysis in Fundulus and other forms studied, or it may be due to differences in technique or in the variety of preparations tested, or other variable experimental conditions.

The comparative studies by Weisel (1948, 1950) may offer a clue to a central theme to unite the diverse findings. In his first investigation he observed the effect of injecting acetone dried teleost pituitary on the melanophores of elasmobranchs, amphibians and reptiles. He found that the extract caused dispersion in all of these. In teleosts, however, aggregation occurred *in vitro* and *in vivo*. Unfortunately *Fundulus heteroclitus* was not included in his material. On this basis he suggested that the chromatophoric hormone was non-specific. His later study did not confirm that suggestion and he was obliged to postulate the existence of two pituitary chromatophorotropic principles, with opposing effects. In his series, the melanophores of all poikilothermic vertebrates, except those he regarded as "higher teleosts", responded to pituitary injection by dispersion. The exceptional "higher teleosts" (e.g. *Fundulus parvipinnis*, *Girella nigricans*, *Gambusia affinis*) showed an aggregation in response to fish pituitary and were indifferent to mammalian pituitary. Weisel (1950) speculated that the divergent responses might be associated with differential sensitivity of the melanophores to the two opposing chromatophoric principles, and that this distinguished higher teleosts from the main line of evolution of cold-blooded vertebrates in which posterior pituitary extract exerts a dispersing effect on melanophores.

While the experiments reported here were not specifically designed to investigate this possibility, they do confirm the *Fundulus* melanophores are essentially insensitive to mammalian posterior pituitary substance *in vitro* and *in vivo*. The methods of separating pituitary hormones developed recently (Burgers, 1961; and Lee *et al*, 1961) should be applied to furnish fractions which could be used to test Weisel's hypothesis.

Although the significance of the pituitary in the background response in Fundulus seems as yet uncertain, the possibility remained that there might be a specific pgimentary hormone of non-pituitary origin. Breder and Rasquin (1950) noted that in certain fish there seemed to be a relationship between phototaxis and the melanophore content of the integument over the pineal region. Species with an exposed pineal region were photopositive; those with a covered region were photonegative. Blinding and covering the pineal region did not completely abolish the reactions to light in such fish suggesting that additional receptors were involved. A review by Rasquin (1958) again drew attention to the probable importance of the pineal in pigmentary responses of teleosts.

Lerner and his co-workers have isolated melatonin from bovine pineal glands and described its structure and some of its functions (1958; 1959a, b; 1960; 1961). No doubt in response to this clue to the hitherto mysterious function of the pineal gland, a large number of papers dealing with melatonin and the pineal soon appeared. Only a few can be mentioned here. Santamarina (1958) found a high concentration of melanin in the pineal glands of castrated bulls, much less in older cows, and none in bulls at any age. He speculated on the possibility of a link between the pineal and the gonads. The work of Wurtman et al (1963) indicated that in rats such a link existed. They found that light inhibited the synthesis of melatonin in the pineal, and that injected melatonin inhibited gonad function. The finding by Wurtman et al (1964) that section of the sympathetic nerves to the pineal abolished the effect of light on the gland suggests that if such a link between the pineal and the gonads exists it is probably very complex.

Weissbach *et al* (1960) and Kopin *et al* (1961) reported on the biosynthesis and metabolism of melatonin in mammals. It was shown that melatonin is synthesized from serotonin in the liver and pineal, final O-methylation taking place in the latter. The hormone is rapidly taken up by all tissues and metabolized in the liver, only a small portion being bound and retained.

Further studies of melatonin by Lerner and Case, (1959c, 1960), Bagnara (1960), and Lerner (1961) showed that it was the most potent agent yet found causing melanin aggregation, especially in frogs. Lerner *et al* (1959) speculated that melatonin might be involved in nervous transmission, based upon their discovery of melatonin in human peripheral nerve fibres. The significance of the observation by Lerner and Case (1959) that a combination of melatonin and acetylcholine caused aggregation *in vitro* in *Fundulus* has yet to be assessed.

The reports cited above suggested that the action of melatonin might serve to explain some of the baffling melanophore responses in *Fundulus*. Accordingly, synthetic melatonin was tested *in vivo*, *in vitro*, and on faded caudal bands, the latter on the supposition that only denervated melanophores might be sensitive to the hormone as they are to intermedin. The results, given in Tables 1, 3, and 4 indicate that under these experimental conditions the melanophores of *Fundulus* are totally indifferent to melatonin. Many repetitions of the experiments confirmed this finding. The very slight dispersion noted in Table 3 was observed infrequently.

It has been shown that as yet there is no convincing evidence for significant hormonal control of physiological colour changes in *Fundulus*, notwithstanding reports to the contrary. Many other teleosts, however, do exhibit a melanophore response to extracts or whole gland preparations

of pituitaries from the same species and from unrelated forms such as mammals.

A comparison of the melanophore responses of fish known to be chromatic to various hormone preparations, drawn from Parker (1948), Pickford and Atz (1957) and Waring (1963), reveals that there is apparently no underlying theme in hormonal control of teleost melanophores. The responses of the melanophores of Ameiurus nebulosus, Anguilla anguilla (vulgaris), Fundulus heteroclitus, Phoxinus laevis, and Parasilurus asotus to what appear to be equivalent preparations may be compared as shown in Table 5.

TABLE 5

Comparison of Teleost Melanophore Responses to Pituitary Hormones

Fish	Preparation	Melanophores		
		Innervated	Denervated	Isolated scale
Phoxinus	Phoxinus whole pituitary	A	A	
	Ameiurus whole pituitary	A	A	
	Intermedin	sl. D		
Parasilurus	Parasilurus whole pituitary	D		A
Ameiurus	Ameiurus whole pituitary	D		
	Phoxinus whole pituitary	O		
	Rana neuro intermediate lobe	D		
	Intermedin	D		
Anguilla	Anguilla whole pituitary	D		
	Intermedin	D		
Fundulus	Fundulus whole pituitary	O	D	A
	Rana neuro intermediate lobe	O	D	O
	Intermedin	O	D	

These data are admittedly scanty. Table 5 includes only the results from experiments in which the conditions and preparations were reasonably equivalent, as far as could be determined. Data from experiments where circumstances were not equivalent or not described in sufficient detail to allow judgement, as well as the results from experiments which were not confirmed by several authors, were discarded. There seems to be no correlation between the importance of hormonal control of melanophores in these fish and their customarily assigned systematic relationships. Within the families grouped together in the Cypriniformes, one gets contrasting situations, i.e. in the minnow (Phoxinus) and the catfishes (Ameiurus and Parasilurus). From the information available Anguilla resembles the catfishes, but the cyprinodont Fundulus apparently differs from all others in showing no response to pituitary hormones by the innervated melanophore. There is obviously a need to subject many other chromatic fish to standardized tests designed to determine whether hormonal control is present and if so how important it is. Until this is done the tentative suggestion by Waring (1963) that there is an evolutionary trend from hormonal to nervous control must be treated with some skepticism.

The question regarding the possible evolution of means of control of colour change in other vertebrates does not seem to have been clearly answered. In cyclostomes the melanophores seem to be under pituitary control and the pineal eye acts as the receptor (Young 1935, Young and Bellerby 1935). No evidence of nervous control was found. The melanophores seemed to respond more to variation in light intensity rather than to background, so that these forms may not be truly chromatic.

Not all elasmobranchs exhibit a background response in a sufficiently short period of time to distinguish it from the slower morphological colour

change. The weight of evidence seems to indicate that the response is under hormonal control, especially if Neill's (1940) criterion is applied, although Parker (1948) cites several of his earlier experiments as giving proof of nervous control to some extent. Three main theories have been advanced to explain colour change in elasmobranchs: (1) the single hormone theory; (2) a dual hormone theory; and (3) a theory postulating nervous control of aggregation in addition to hormonal control of dispersion. Fingerman (1963 and 1965) presents the evidence for these hypotheses. Much of the discussion has centered around the dual hormone theory of Hogben (1936) and his co-workers (Waring, 1963), but Mellinger (1963) has presented histophysiological grounds for rejecting this theory, at least for Scyliorhinus caniculus. Parker (1935a, c) and Vilter (1937) were the chief exponents of nervous control. There has been no recent work on these lines, so that the importance of nervous control is still undecided.

The earlier part of this discussion on comparative relationships reviewed chromatic control in teleosts especially in relation to the results on Fundulus heteroclitus reported here. One might extend that discussion to include the control of colour change in teleost flatfishes e.g. Pleuronectidae, in which control is mainly if not exclusively nervous (Waring 1963). However, flatfishes show the peculiar phenomenon of pattern reproduction and this aspect of the problem will be discussed later.

In Amphibia, there is almost unanimous agreement that colour change is under pituitary control (Parker, 1948; Waring, 1963; Fingerman, 1965). Allen (1916) and Smith (1916), independently, first observed that in Rana larvae hypophysectomy resulted in permanent paling. Atwell (1919)

showed that this was due to persistent aggregation of melanin, followed later by pigment loss.

Hogben and Winton (1916, 1922) evoked dispersion of the melanin in frog melanophores with extracts of the pituitaries of fish, amphibians, reptiles, birds and mammals. The dispersing hormone was most concentrated in the posterior lobe. So reliable is this response that it is the basis of a number of methods for detecting and assaying melanophorotropic hormone (Wright, 1954; Roggen, 1962). The hormone is usually referred to as intermedin although many synonyms are still in common use. A review of the literature suggests that the degree of background adaptation varies with the habitat of the amphibian, being slight or absent in more terrestrial forms. The significance of this interesting trend, which should be substantiated, may be ecological, or it may be related to a general evolutionary adaptation. This will be discussed later.

The following quotation from Parker (1948, p. 208) summarizes the opinion held generally of the means of control of melanophores in Amphibia: "Amphibian colour changes are induced primarily by a neuro-humor [=hormone] from the intermediate lobe of the pituitary gland, intermedine, whose disappearance from the blood of the given amphibian induces blanching." The adequacy of this statement which relegates nervous control to a position of very minor importance if not denying it completely, has been demonstrated many times and was elaborated by Burgers et al (1963). These workers found a higher concentration of melanophore-stimulating hormone (MSH) in the pituitary glands of Xenopus laevis when the animal was on a white background than when it was on a black background. This suggests that the dispersing hormone is produced continuously but released only in response to a dark background.

While the evidence cited above provides a satisfactory understanding of colour change in amphibians, there is some evidence that more than one hormone may be involved. Brick *et al* (1959) showed that the skin of *Ambystoma maculata* larvae contained an MSH-like substance. The powerful aggregation of melanin by adrenaline has been demonstrated frequently. A more specific agent for bringing about blanching was demonstrated by Bagnara (1960). He showed that in the larvae of *Xenopus* aggregation of melanin may be mediated by the pineal gland because blanching in the dark could be abolished by pinealectomy. Replacement therapy restored the reaction. Serotonin, a dispersing agent, was found in the skin secretion of *Xenopus* (van de Veerdonk 1960). Zimmerman and Dalton (1961), using cultured embryonic tissue of *Amblystoma*, *Triturus* and *Siredon in vitro*, and *Amblystoma in vivo*, obtained marked reactions to adrenaline, noradrenaline, ACTH and acetylcholine. The first two agents caused aggregation, the latter two dispersion. Eserine was applied prior to acetylcholine to obtain dispersion. In contrast, Wright and Lerner (1960) found acetylcholine ineffective *in vitro*. It might be surmised that the ACTH may have contained significant MSH contamination (Reinhardt *et al* 1952). However, Chavin (1956) showed that ACTH and MSH are not equivalent, using the fish *Carassius* as the test animal. Dixon (1956) demonstrated that corticotrophin has slight intrinsic MSH activity *in vitro* and Steelman and Guillemin (1959) observed that α-MSH has ACTH activity. Finally, Sulman (1952) obtained dispersion of melanin in *Hyla arborea* with ACTH.

It seems clear, therefore, that while the importance of intermedin (=MSH) in amphibian colour change has been amply substantiated, the validity of the one hormone theory has not.

Despite the accumulation of an extensive literature on reptilian colour change, it is difficult to propose a hypothesis sufficiently general to cover all known instances. It may be tentatively stated that in older primitive groups colour change is under predominantly hormonal control, while nervous control is dominant in more highly specialized groups such as chameleons. This view is based on experimental evidence presented by many authors (Redfield, 1916; Hogben and Winton, 1916, 1922; Hadley, 1931; Rahn, 1941; Weisel, 1948, 1950; Novales, 1959; Burgers, 1961), on the discussions in the reviews by Parker (1948), Waring (1963), Fingerman (1965) and especially on the time relations of the background responses of different reptiles.

Physiological colour change is confined to poikilothermous animals, but morphological colour change in which there is a change in the number of melanophores and the amount of melanin in the skin, is not so restricted. Birds and mammals possess the components for physiological colour change, but the coverings of feathers and hair rule out the possibility of widespread occurrence of physiological colour change in warm-blooded vertebrates.

When the occurrence and distribution of the physiologically active substances e.g. biogenic amines, are studied it becomes apparent that they are very widespread amongst different kinds of animals. The functions which they serve depend more on the evolution of target organs capable of responding specifically to them than to the evolution of new and peculiar substances in higher groups.

It is interesting that melanophores are capable of giving a specific response to substances occurring in the urine of patients suffering from endocrine disorders. Durlach (1959) has proposed this response as a means of carrying out differential diagnosis.

3. Comments on the melanophore as an active cell

Before discussing other aspects of colour change, it may be useful to mention some of the more recent findings concerning the melanophore and its pigment, melanin.

The vertebrate melanophore conforms to the customary histological description of a cell, though the stellate shape distinguishes it from most cells in appearance, giving it some features in common with nerve cells and other cells with ramifying processes. Of course its unique feature is the possession of melanin as a normal cytoplasmic inclusion. The physical basis for the movement of melanin granules will be touched upon later.

The embryonic origin of melanophores was a matter of some dispute (Spaeth, 1916a; Lopashov, 1944; Rawles, 1948; Newth, 1951; Orton, 1953; Okun, 1965), but the conclusion of Lopashov that they are neural crest derivatives seems to be well established (Hörstadius, 1950; Lehman and Youngs, 1952; Lerner, 1959; Silvers, 1961). Waring (1963) cites work indicating that all chromatophores have a common origin.

Melanophores may exhibit amoeboid movement in pre-adult stages (Gilson 1926) and possibly thereafter (Caldwell and Caldwell 1962). In general, however, most reports state that in Fundulus the cell border of the mature melanophore is fixed (Matthews 1931).

A detailed discussion of melanin and melanogenesis cannot be given here. The chemistry and biosynthesis of the pigment is treated by Rangier (1960) and Swan (1963), and some aspects of melanogenesis may be found in Osborn (1941), Pickford (1957), Hu and Chavin (1960), and Delage and Porte (1963). It is interesting that some of the same molecular "building bricks" are involved in melanin synthesis e.g. tyrosine, as in the

biosynthesis of the catechol amines adrenaline and noradrenaline. However, this may be fortuitous.

Fänge's (1962) review includes most of the data available on the pharmacology of melanophores. Sawyer et al (1961) provide a good discussion of the possible significance of the effects on melanophores of the various neurohypophyseal extracts from fish. The papers by Robinson and Scott (1960), Khazan and Sulman (1961) and Scott (1962) may be mentioned as indicating a recent trend towards the use of melanophores in investigating the site and mechanism of drug action.

There has been much discussion about the possible physical processes involved in the movement of melanin granules. It is generally believed that movement is triggered at the membrane of the melanophore, by chemical transmitters or hormones, although Pouchet (1871b) found nerve fibres in direct contact with chromatophores in a gold-impregnated preparation of the pectoral fin of a young flatfish. He believed that the nerve fibre penetrated the cell. There seem to be no further reports of similar findings and bearing in mind that it was an early observation it may be assumed that the active agent diffuses across the neuro-effector junction in the case of innervated chromatophores, or through the tissue fluid in the case of chromatophores under hormonal control.

The question of the changes which these agents bring about at the membrane has been under investigation recently. Novales (1959, 1962) showed that Na^+ was essential for the action of MSH in frog skin. He suggested that MSH might regulate the permeability of the cell membrane to Na^+, or possibly affect the Na^+ pump. When Na^+ concentration in the cell rose dispersion occurred. MSH without Na^+ caused aggregation, indicating that MSH does not cause dispersion directly. Because Ca^{++}

has a basic role in membrane reactions, Dikstein et al (1963a, b) believed that MSH action should not be strictly Na^+ dependent. They showed that Ca^{++} could substitute for Na^+, even after the melanophore membrane was depolarized by K^+. They concluded that Ca^{++} is the coupling agent for MSH action and melanin dispersal. Dikstein and Sulman (1964), again using Hyla arborea skin, found that dispersion rate was not temperature dependent (range 13°-20° C) but dispersion was inhibited at 37° C. In contrast the aggregation rate increased as temperature increased. They proposed the hypothesis that the passive state, dispersion, was induced by the entrance of Ca^{++} into the cell and the exit of K^+. The active state, aggregation required energy to pump out Ca^{++} or to bring K^+ in across the cell membrane. The hypothesis may be considered in the light of reports by Lerner et al (1954) that MSH action on frog skin is anaerobic in vivo but not in vitro (Lerner and Takahashi, 1956; Lerner and Case, 1959). It seems possible to combine these findings by clearly defining the energy sources for melanin movement, as suggested by Dikstein and Sulman.

It is interesting that certain experiments described in the present work indicated that movement of melanin granules in Fundulus is not Ca^{++} dependent, in contrast to the above reports on Amphibia.

4. The functional role of adrenergic mechanisms

Von Euler (1951) pointed out that in mammals, the main function of noradrenaline is control of the circulation under normal circumstances, while adrenaline acts to bring about an integrated response to states of stress and emergency. Noradrenaline is the chief adrenergic nerve mediator, and adrenaline acts more like a true hormone.

Can these concepts be applied to the proposed adrenergic mechanism of colour control in Fundulus heteroclitus? The anatomical features of the autonomic nervous system in teleost fish suggest that, by analogy with mammals, both divisions of the autonomic nervous system are present, and that there is autonomic innervation of the skin (Romer 1963). Von Euler (1953) demonstrated the presence of adrenaline and noradrenaline, which he took as evidence of chromaffin tissue or adrenergic innervation, in the viscera of selachians and teleosts. Adrenaline was more abundant that noradrenaline, in contrast to mammals, but neither was as abundant as in the latter group. On this and additional evidence von Euler was led to suggest that the unmyelinated fibres leaving the sympathetic ganglia are adrenergic and that teleosts possess a true sympathetic nervous system.

If these be granted, certain suggestions may be made. It appears that a dual autonomic nervous system may be phylogenetically more ancient than previously supposed. Perhaps in lower forms with rapid background adaptation the process is mediated wholly by an adrenergic mechanism. In higher forms Parker (1948) has shown that denervation of an area of skin in Mustelus and Squalus results in a fairly rapid blanching of the area, suggesting that in these species at least, nervous control of the melanophores may be present. The operations were done without disturbing the blood supply to the denervated areas. In higher forms with relatively slower responses the adrenergic function might be withdrawn and applied to other processes, while control of the chromatophores came secondarily under hormonal control. It should be noted that this suggestion is in direct opposition to Waring (1963) who postulated an archaic pituitary control with nervous control acquired secondarily. The suggestion outlined here may serve to

harmonize, at least in part, the means of control with the systematic position of a chromatic vertebrate. At any level of organization the predominance of one or the other means of control would reflect the animal's environmental circumstances rather than its phylogeny. The argument may appear tinged with Lamarckianism, yet if two potentially adequate systems of control are available, it does not seem wholly unreasonable to suppose that the notorious parsimony of nature will subvert one to other functions as environmental circumstances require.

The suggestion is made more attractive by the following considerations. It appears that all the anatomical components of an adrenergic pigmentary effector system have a common embryonic origin (Hörstadius 1950). There is considerable evidence of a fairly close biochemical relationship among adrenaline, noradrenaline, melanin, serotonin, melatonin, and possibly MSH, as well as their precursors. Furthermore, MSH is present in higher, non-chromatic vertebrates (Waring and Landgrebe, 1949; Ivy and Albert, 1957; Dixon and Li, 1961) including primates (Varon, 1959; Karkun et al, 1960; Lee et al, 1961). The normal function of MSH in these forms is not known, but over production results in increased pigmentation (Karhausen et al 1959). A hint is given in Courrier and Čehović (1960). They found that α-MSH depressed thyroid activity in the rabbit. Another example of an unsuspected funtion of a hormone is the observation of Pickford and Phillips (1959) that prolactin helps to prolong survival of hypophysectomized Fundulus in fresh water.

Further support is given by the repeated observation that hypophysectomy usually results in profound pigmentary changes whether the animal is chromatic or not. The rate at which the change occurs seems to be related to the dominant means of control in chromatic animals.

A similar evolutionary hypothesis has been discussed in Section 2.

Both the hormonal and adrenergic mechanism of control are intimately involved in other functions in the body other than colour change, even in animals where one or both of them play some part in controlling this phenimenon. Therefore the roles of these components in colour change cannot be examined in isolation from their roles in the body as a whole. The primary distinction between the two mechanisms in colour change is a temporal one. The slower and more persistent colour change phenomena are usually controlled by hormonal mechanisms of some kind. The more rapid and more easily reversible colour changes come under the control of the autonomic system. In these cases where this is carried out by the sympathetic system, as seems to be true in Fundulus the adrenergic mechanism is performing its familiar role assisting the body in its general response to stress and environmental changes which call for a rapid response on the part of the animal. For example, the potentially dangerous environmental change presented by a sudden increase in illumination leading to an increased albedo and making the fish more conspicuous, evokes paling. This tends to make the fish less conspicuous.

5. Some unsolved problems of pigmentary effectors

It seems probable that with the use of both traditional and more modern methods of investigation the means of control of colour change in chromatic vertebrates can be determined. Impressive progress has already been achieved in fish (Rasquin 1958) and in many other forms (Fingerman 1965).

But many questions remain without a completely satisfactory answer. Among these, are (1) the phenomenon of the formation, fading and revival of the caudal band; (2) the mechanism of melanin movement; and (3) pattern

reproduction in flatfishes.

(a) Caudal bands - If Parker's (1948) dual innervation hypothesis is true, the formation of the caudal band is explained on the basis of differential sensitivity of the two types of nerves to the stimulus of the initiating cut. As the band persists for such a long time it is necessary to admit that the impulses arising from potential injury in the dispersing fibre are also persistent, and further, that the fibre remains functional for an extraordinarily long time after its connection with its cell body has been severed. Such properties are rare if not unique, yet Abramowitz (1935) based his experiments on the rate of nerve regeneration in Fundulus heteroclitus on them.

The question seems to be: what is the physiological effect of the initiating cut? Parker (1934b) concluded from his cold block experiments that caudal bands were formed by an active process and not by releasing the melanophore from a central aggregating control.

Fading of the caudal band, according to the Parker hypothesis, is brought about by the diffusion of an aggregating substance (neurohumor) from the surrounding innervated tissue into the band. This was supported by Mills' (1932a) observation that aggregation of the melanin in the band area commenced at the periphery. It is unlikely that fading is due to the activity of nerve fibres from the surrounding area because chromatic nerve fibres in the caudal fin follow an essentially straight course parallel to the fin rays with very few short lateral branches (Mills 1932b). The diffusing neurohumor may not be similar or identical to known chemical transmitters, for it is an essential property of the latter that they be capable of undergoing rapid inactivation. That this is probably not the case for the hypothetical neurohumors is shown by the following experiment (Parker 1934b, 1935b). If a caudal band is

formed and caused to fade on an illuminated white background, and the fish is then placed on an illuminated black background, the band will appear as a pale stripe while the rest of the fish rapidly darkens. The pale stripe slowly becomes dark if the fish is maintained on the latter background. If the fish is then transferred to a white background and maintained on it, the band appears as a dark stripe which slowly fades. The slowness with which the band comes to match the body of the fish after such background exchanges is in marked contrast to the speed of normal background responses which are very nearly complete in two minutes (Parker 1948). The question of the identity of the neurohumors of Parker with chemical transmitters is certainly still open, although the aggregating effects of MAOI *in vivo* and upon caudal bands reported here (Tables 1 and 4) may be indicative.

(b) Mechanism of melanin movement - The means by which pigment is caused to migrate within the chromatophore, as distinct from the mechanisms of control of the colour change which the migration brings about, has received attention ever since chromatophores were first described (Sangiovanni, cited in Parker, 1948). Matthews (1931) showed that the cell borders of the melanophore were fixed, and concluded that migration of pigment was probably associated with physical changes in the cytoplasmic colloid and not as a consequence of amoeboid movement, confirming a suggestion by Spaeth (1916). Marsland (1936, 1944, 1948) showed by means of experiments under high hydrostatic pressure that aggregation of melanin was associated with gelation, and dispersion with solation of the cytoplasm. Kamada and Kinosita (1944) observed that melanin granules in an isolated branch of a melanophore were capable of movement, and that granules moved at a constant individual rate. They also noted changes in the volume of the centrosphere as aggregation or

dispersion took place. Using frog melanophores Wright and Lerner (1960) observed cytoplasmic streaming when melanin granule movement occurred, accompanied by ion exchange across the cell membrane. The work cited above provides evidence for what might be termed the colloidal hypothesis of pigment migration.

A second hypothesis, which might be called the electrophoretic or ionic hypothesis, has been set out by Kinosita (1963). He employed an extremely delicate microtechnique on fish melanophores and found that there was a radial gradient of electric potential from the centrosphere to the branches of the cell. Melanin granule movement corresponded to changes in the potential. Kinosita concluded that the movement of the granules migrated electrophoretically along the potential gradient. Support for this theory was offered by Nagahama (1953), Novales (1959), and Dikstein (1963a, b; 1964), using melanophores from Hyla arborea.

A suggestion by Falk and Rhodin (1957) based upon electron microscopic observations of melanophores in scales from Lebistes reticulatus, is at variance with both the hypotheses discussed above, and forms the basis of what might be called the mechanical theory of melanin movement. These workers found a zone between an inner and an outer cytoplasmic membrane which was occupied by a mesh-work of fine fibrils apparently unattached to either membrane and showing a preferred orientation parallel to the membranes. The width of the zone varied with pigment dispersion, from about 0.5μ in fully dispersed melanophores to about 3μin fully contracted melanophores. From these observations they suggested "that the migration of the pigment granules within the teleost melanophores should be due to contracting and relaxing fibrils in the zone between the two membranes." (p. 215)

Novales (1962) reviews and discusses these problems in pigment granule migration. The evidence on which the three hypotheses are based seems reliable. It may be possible, with difficulty, to bring the first and second into agreement. As far as is known, no attempt has been made to provide experimental evidence which would combine all three.

(c) Pattern reproduction in flatfishes - A unique problem is presented by the chromatic behaviour of flatfish. These fish are capable of conforming to both colour and pattern of the background (Mast, 1916). The mechanism of control may be similar to that in Fundulus because the eyes are essential, denervation causes dispersion, adrenaline causes paling, and flatfish melanophores are insensitive to pituitary hormones (Osborn, 1939). More basic to the problem are questions concerning the relation of the retinal receptors to the effectors. How is information about colour and pattern received at the retina, coded, and translated into a reproduction of the colour and pattern by the melanophores? The accuracy of the reproduction suggests almost a 1:1 relation between receptor and effector, which seems anatomically impossible. In what form then does the information reach the melanophores? Parker (1948) invoked nervous and hormonal control to suit the circumstances. Waring (1963), more conservatively, was of the opinion that it "...is a complete mystery at present..." (p. 102). Significant work on the problem, since that of Osborn noted above, does not seem to have been done.

6. Ecological role of colour change in Fundulus heteroclitus

In carrying out the experiments described above, and in so testing Fundulus, a number of casual observations were made which throw some light on the ecological role of colour change in this fish.

Fingerman (1965) lists the functions of colour change as: Protective colouration; thermoregulation; mating and associated displays.

For Fundulus, the first and last are probably important. It seems clear that temperature control is not a problem for fish because of the high thermal capacity of water. In addition, Fundulus appears to be eurythermal, passing in nature from surface water at about 30° C to deeper levels at about 20° C or less with no sign of distress. In the laboratory it is possible to alter water temperatures through a wide range without danger to the fish. Except when the lower and upper limits are approached, below 5° C and above 25° C respectively, no alteration of colour is apparent (Cole and Schaeffer 1937).

Fundulus males exhibit striking colours associated with breeding activities, as do many other fish. These changes however are probably not related to the background adaptation reaction (Newman 1918) and may be omitted from the discussion.

Protective colouration may be considered as having two aspects, that of rendering the prey less visible to a predator, and the converse. In the case of Fundulus the latter is most probably the important function of colour change, because the only known predator of Fundulus in the area from which fish were supplied is the relatively sluggish bottom-dwelling Opsanus tau (Schwartz 1964).

Fundulus heteroclitus is a surface feeding fish in nature, but during the course of this work observed fish were in captivity feeding at all levels including the bottom. Floating or sinking food was, however, preferred.

Mixed schools of fish frequently occurred, predominantly Fundulus heteroclitus, but including F. majalis, F. diaphanus and Cyprinodon variegatus. F. diaphanus appeared to have almost comparable background adaptation to F. heteroclitus. F. majalis had little or no adaptation. The background adaptation capability of Cyprinodon variegatus was difficult to assess as they were in breeding colours, but some adaptation

was evident in the females. As the role of colour change in Fundulus heteroclitus seems to be primarily concealment from prey, the association with less chromatic fish would seem to be beneficial only to the latter.

It is generally agreed that background adaptation enhances the survival of both predator and prey. Young (1916) after testing a number of predator-prey pairs was led to conclude that immobility was more important than colouration in protecting prey from predators. His conclusion has not been supported by later workers. Sumner (1935a), using Gambusia as prey and wading and diving birds as predators, showed that more fish were taken when they contrasted with the bottom. Using background-adapted Gambusia again and fish predators, Sumner (1935b) obtained similar results and concluded further that immobility was a disadvantage under the circumstances. These and other experiments are discussed and summarized by Sumner (1945). It is possible that the artificial conditions of these experiments may bias the results and that the situation in nature may be quite different to that suggested by Sumner.

In his review Hogben (1942) noted the probability that background adaptation has survival value, if only because it is so widespread. Most chromatic animals are aquatic and for these the function may be protective or concealing. Terrestrial chromatic animals are poikilothermous, suggesting that colour change in these forms may be primarily linked with thermoregulation, with camouflage and sexual displays of secondary importance. A considerable body of literature has grown up on the question of the relative importance of these three functions of colour change in terrestrial animals, but it is nevertheless difficult to form a general conclusion.

An interesting example of camouflage in fish, involving both colour change and pattern was described by Breder and Rasquin (1955). Chaetodipterus is normally banded in turbid water or against a mottled background, at low to moderate light intensities. At high light intensity and in clear water the fish become dark. The authors state that in this condition the fish closely resemble bottom litter. They found that the injection of adrenaline caused generalized darkening and partial obliteration of the barred pattern.

Although it appears obvious that the background colour response has survival value in those animals which display this phenomenon, the precise nature of this adaptation in an ecological sense still remains to be worked out in many cases. Most of our knowledge of the phenomenon of colour change is based on laboratory experiments carried out under artificial conditions which animals would never meet in nature. Thus fishes would probably never encounter completely white or black backgrounds, yet most of the experiments have been carried out in these situations. There is a need for extending the studies of colour change to organisms in their natural surroundings as well as continuing work to analyse and define more precisely the physiological mechanism of this remarkable activity.

V. CONCLUSIONS

1. Catechol amines and related substances cause aggregation of melanin granules in melanophores both *in vivo* and *in vitro*. It is concluded that an adrenergic aggregating mechanism exists in *Fundulus*.

2. Similar experiments with acetylcholine and related substances gave no evidence of a cholinergic dispersing mechanism. It is concluded that a cholinergic dispersing mechanism is absent in *Fundulus*.

3. Experiments with biogenic substances other than catechol amines did not yield conclusive results. It may be concluded that these substances do not play a major part in physiological colour change in *Fundulus*.

4. Substances which, in mammals, block the sympathetic nervous system at various levels usually cause dispersion *in vivo*. Some, however, also cause dispersion *in vitro*, suggesting that they have a local as well as a systemic action. The evidence from experiments using sympathetic blocking agents supports the first conclusion.

5. Atropine causes dispersion *in vivo* at high doses, but is ineffective *in vitro*. Dispersion *in vivo* after atropine is probably due to ganglion blockade by the drug, and it is therefore concluded that this result supports the second conclusion.

6. Substances injected into fish with fresh and faded caudal bands usually caused changes in the caudal bands consistent with their

effects in intact fish. Exceptions to this statement were found, however, and no general conclusion can be drawn from the results of these experiments.

7. The removal of Ca^{++} in isolated scales by treatment with a chelating agent (EDTA) does not abolish aggregation or dispersion in response to appropriate drugs. It is concluded that calcium is not essential for the movement of melanin granules.

8. The melanin of melanophores in isolated scales from Fundulus is aggregated by heat and by cold. Light has no effect. It is concluded that light is not an adequate stimulus for melanophores acting as independent effectors.

VI. LITERATURE CITED

Abramowitz, A.A. 1935. Regeneration of chromatophore nerves. Proc. Nat. Acad. Sci. 21: 137-141.

Abramowitz, A.A. 1937. The role of the hypophyseal melanophore hormone in the chromatic physiology of Fundulus. Biol. Bull. 73: 134-142.

Allen, B.M. 1916. The results of extirpation of the anterior lobe of the hypophysis and of the thyroid of R. pipiens larvae. Science 44: 755-758.

Atwell, W.J. 1919. On the nature of the pigmentation changes following hypophysectomy in the frog larva. Science 49: 48-50.

Bagnara, J.T. 1960. Pineal regulation of the body lightening reaction in amphibian larvae. Science 132: 1481-1482.

Bainbridge, J.G. and D.M. Brown. 1960. Ganglion-blocking properties of atropine-like drugs. Brit. J. Pharmacol. 15: 147-151.

Bein, H.J. 1960. Some pharmacological properties of guanethidine. in CIBA Symposium on Adrenergic Mechanisms: 162-172.

Boura, A.L.A. and A.F. Green. 1959. The actions of bretylium; adrenergic neurone blocking and other effects. Brit. J. Pharmacol. 14: 536-548.

Boura, A.L.A., F.C. Copp, W.G. Duncombe, A.F. Green, and A. McCoubrey. 1960. The selective accumulation of bretylium in sympathetic ganglia and their postganglionic nerves. Brit. J. Pharmacol. 15: 265-270.

Boyd, H., V. Chang and M.J. Rand. 1960. The anticholinesterase activity of some antiadrenaline agents. Brit. J. Pharmacol. 15: 525-531.

Breder, C.M. and P. Rasquin. 1950. A preliminary report on the role of the pineal organ in the control of pigment cells and light reactions in teleost fish. Science 111: 10-12.

Breder, C.M. Jr. and P. Rasquin. 1955. Further notes on the pigmentary behaviour of Chaetodipterus in reference to background and water transparency. Zoologica 40: 85-90.

Brick, I.H., Clark Dalton and G.L. Vankin. 1959. A melanophore-stimulating factor in embryonic skin. Proc. Soc. Exptl. Biol. Med. 102: 657-659.

Burgers, A.C.J. 1961. Occurrence of three electrophoretic components with melanocyte-stimulating activity in extracts of single pituitary glands from ungulates. Endocrinology 68: 698-703.

Burgers, A.C.J., K. Imae and G.J. van Oordt. 1963. The amount of MSH in single pituitary glands of Xenopus laevis kept under various conditions. Gen. Comp. Physiol. 3: 53-57.

Burn, J.H. 1950. Relation of motor and inhibitor effects of local hormones. Physiol. Revs. 30: 177-193.

Burn, J.H. 1963. The Autonomic Nervous System. Blackwell Scientific Publications, Oxford.

Burn, J.H. and M.J. Rand. 1958. The action of sympathomimetic amines in animals treated with reserpine. J. Physiol. Lond. 144: 314-336.

Burn, J.H. and M.J. Rand. 1960. Sympathetic postganglionic cholinergic fibres. Brit. J. Pharmacol. 15: 56-66.

Caldwell, M.C. and D.K. Caldwell. 1962. Suspected melanophore movement in fishes beyond the larval stage. Bull. S. Calif. Acad. Sci. 61: 129-132.

Chavin, W. 1956. Pituitary-adrenal control of melanization in xanthic goldfish, Carassius auratus L. J. Exp. Zool. 133: 1-45.

Cole, W.H. and K.F. Schaeffer. 1937. The effect of temperature on the rate of adaptation of Fundulus to black and white backgrounds. Bull. Mt. Desert. Isl. Biol. Lab.: 26-30.

Courrier, R. and G. Čehović. 1960. Action de l'hormone mélanophorotrope purifiée (∝-MSH) sur la fonction thyroidienne chez le Lapin. C. R. Acad. Sci. (Paris) 251 (1): 832-834.

Delage, J. and A. Porte. 1963. Etude au microscope électronique de la mélanogenèse dan des mélanocytes de Poisson. Vol. D. Bull. Inst. Oceanog. Monaco. ID.: 117-120.

Deutsch, S., E. Angelakos and E.R. Loew. 1956. A quantitative method for measuring melanophore expanding activity. Endocrinology 56: 33-39.

Dikstein, S. 1963. Mechanism of melanophore reaction. Bull. Res. Counc. Israel 10E: 221.

Dikstein, S. and F.G. Sulman. 1964. Mechanism of melanophore dispersion. Biochem. Pharmacol. 13 (6): 819-826.

Dikstein, S., C.P. Weller and F.G. Sulman. 1963. Effect of calcium ions on melanophore dispersal. Nature 200 (4911): 1106.

Dixon, H.B.F. 1956. The melanophore-stimulating activity of corticotrophin. Biophys et Biochim Acta 19: 392-394.

Dixon, J.S. and C.H. Li. 1961. The isolation and structure of β-MSH from horse pituitary glands. Gen. Comp. Endocrinol. 1: 161-169.

Durlach, J. 1959. Test de l'écaille du cyprin bronzé; nature et rôle dans la mélanogenèse, du principe mélanotrope hypophysaire. Ann. Endocrinol. T.20(1): 31-42.

von Euler, U.S. 1951. The nature of adrenergic nerve mediators. Pharmacol. Revs. 3: 247-277.

von Euler, U.S. 1953. Presence of catecholamines in the visceral organs of fish and invertebrates. Acta Physio. Scand. 28: 297-305.

Falk, S. and J. Rhodin. 1957. Mechanism of pigment migration within teleost melanophores. Proc. First Eur. Reg. Conf. Elect. Micros. (Stockholm): 213-215.

Fänge, R. 1962. Pharmacology of poikilothermic vertebrates and invertebrates . Pharm. Rev. 14: 281-316.

Fingerman, M. 1963. The Control of Chromatophores. Pergamon Press.

Fingerman, M. 1965. Chromatophores. Physiol. Revs. 45: 296-339.

von Frisch, K. 1911. Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. Pflügers Arch. f. d. ges. Physiol. 138: 319-387.

Fujii, R. 1960. The seat of atropine action in the melanophore dispersing system of fish. J. Fac. Sci. Univ. Tokyo 8: 643-657.

Gilson, Jr., A.S. 1926 . The control of melanophore activity in Fundulus. J. Exp. Zool. 45: 457-468.

Ginsburg, J. and A.F. Cobbold. 1960. Effects of adrenaline, noradrenaline and ispropylnoradrenaline in man. m: Adrenergic Mechanisms, J. and A. Churchill, Ltd., London.

Goodman, L.S. and A. Gilman. 1965. The Pharmacological Basis of Therapeutics. (3rd ed.) The Macmillan Co., N.Y.

Gray, E.G. 1956. Control of the melanophores of the minnow (Phoxinus phoxinus [L.]). J. Exp. Biol. 33: 448-459.

Hadley, C.E. 1931. Color changes in excised and intact reptilian skin. J. Exp. Zool. 58: 321-331.

Healey, E.G. 1940. Über den Farbwechsel der Elritze (Phoxinus laevis Ag.). Zeitschr. verg. Physiol. 27: 345-586.

Healey, E.G. 1948. The colour change of the minnow (Phoxinus laevis Ag.). Bull. Anim. Behav. 6: 5-15.

Healey, E.G. and D.M. Ross. 1966. The nervous control of the background colour response in the European minnow Phoxinus phoxinus L. Comp. Biochem. and Physiol. (in the press).

Hill, A.V., J.L. Parkinson and D.Y. Solandt. 1935. Photo-electric records of the colour change in Fundulus heteroclitus. J. Exp. Biol. 12: 397-399.

Hogben, L. 1924. The Pigmentary Effector System. Oliver and Boyd, Edinburgh.

Hogben, L.T. 1936. The pigmentary effector system. VII. The chromatic function in elasmobranch fishes. Proc. Roy. Soc. B 120: 142-158.

Hogben, L. 1942. Chromatic behaviour. Proc. Roy. Soc. B. 131: 111-136.

Hogben, L. and D. Slome. 1931. The pigmentary-effector system. VI. The dual character of endocrine coordination in amphibian colour change. Proc. Roy. Soc. B, 108: 10-53.

Hogben, L. and F.R. Winton. 1916. Studies on the pituitary. I. The melanophore stimulant in posterior lobe extracts. Biochem. J. 16: 619-630.

Hogben, L.T. and F.R. Winton. 1922. LXII. Studies on the pituitary. 1. The melanophore stimulant in posterior lobe extracts. Biochem. J. 16: 619-630.

Hörstadius, S. 1950. The Neural Crest. Oxford University Press.

Hu, F. and W. Chavin. 1960. Hormonal stimulation of melanogenesis in tissue culture. J. Invest. Dermatol. 34: 377-391.

Ivy, H.K. and A. Albert. 1957. Attempts to alter the concentration of melanophore hormone in the pituitary gland of the rat. Endocrinology 61: 667-675.

Jenkin, P.M. 1962. Animal Hormones. Part 1. Pergamon Press.

Kamada, T. and H. Kinosita. 1944. Movement of granules in fish melanophores. Proc. Imp. Acad. Jap. 20: 484-492.

Karhausen, L.R., R.F. Tagnon, J. Brihaye, S. Zylberszac, and J. Henneaux. 1959. Hypopituitarism and skin pigmentation associated with malignant hypertension and an intrasellar cholesteatoma. Cancer. 13: 527-536.

Karkun, J.N., F. W. Landgrebe, R.A. Main and G.M. Mitchell. 1960. Melanophore-expanding substances in human blood. J. Physiol. 152: 459-466.

Kent, A.K. 1959. Distribution of the melanophore aggregating hormone in the pituitary of the minnow. Nature 183: 544-545

Khazan, N. and F.G. Sulman. 1961. Melanophore-dispersing activity of reserpine in Rana frogs. Proc. Soc. Exptl. Med. Biol. 107: 282-284.

Kinosita, H. 1963. Electrophoretic theory of pigment migration within fish melanophore. Ann. N. Y. Acad. Sci. 100: 992-1003.

Kleinholz, L.H. 1935. The melanophore-dispersing principle in the hypophysis of Fundulus heteroclitus. Biol. Bull. 69: 379-390.

Kopin, I.J., J. Axelrod and E. Gordon. 1961. The fate of melatonin in animals. J. Biol. Chem. 236: 3072-3075.

Lee, T.H., A.B. Lerner, and V. Buettner-Janusch. 1961. The isolation and structure of α and β MSH from monkey pituitary glands. J. Biol. Chem. 236(5): 1390-1394.

Lehman, H.E. and L.M. Youngs. 1952. An analysis of regulation in the amphibian neural crest. J. Exp. Zool. 121: 419-443.

Lerner, A.B. 1959a. Mechanism of hormone action. Nature 184: 674-677.

Lerner, A.B. 1959b. Melatonin in peripheral nerve. Nature 183: 1821.

Lerner, A.B. 1961. Hormones and skin color. Scientific American 205: 98-108.

Lerner, A.B. and J.D. Case. 1959. Pigment cell regulatory factors. J. Invest. Dermatol. 32: 211-221.

Lerner, A.B. and J.D. Case. 1960. Melatonin. Fed. Proc. 19: 590-592.

Lerner, A.B., J.D. Case, Y. Takahashi, T.H. Lee and W. Mori. 1958. Isolation of melatonin the pineal gland factor that lightens melanocytes. J. Am. Chem. Soc. 80: 2587.

Lerner, A.B., K. Shizume and I. Bunding. 1954. The mechanism of endocrine control of pigmentation. J. Clin. Endocrinol. and Metab. 14: 1463-1490.

Lerner, A.B. and Y. Takahashi. 1956. Hormonal control of melanin pigmentation. Recent Progress in Hormone Research 12: 303.

Long, J.M. and R. Guillemin. 1961. On the *in vitro* bio-assay for measuring melanophoretic activity. Experientia 17: 132-134.

Lopashov, G.V. 1944. Origin of pigment cells and visceral cartilage in teleosts. C. R. Acad. Sci. U.R.S.S. 44(4): 169-172.

Marsland, D.A. 1944. Mechanism of pigment displacement in unicellular chromatophores. Biol. Bull. 87: 252-261.

Marsland, D. 1948. Protoplasmic contractility. Sci. Month. 67: 193-200.

Marsland, D.A. and D.E.S. Brown. 1936. Amoeboid movement at high hydrostatic pressure. J. Cell and Comp. Physiol. 8: 167-178.

Mast, S.O. 1916. Changes in shade, color, and pattern in fishes and their bearing on the problems of adaptation and behaviour, with

especial reference to the flounders Paralichthys and Ancylopsetta. Bull. U. S. Bur. Fish. 34: 173-238.

Matthews, S.A. 1931. Observations on pigment migration within the fish melanophore. J. Exp. Zool. 58: 471-486.

Matthews, S.A. 1933. Color changes in Fundulus after hypophysectomy. Biol. Bull. 64: 315-320.

Mellinger, J. 1963. Etude histophysiologique du système hypothalamo-hypophysaire de Scyliorhinus caniculus (L.) en ètat de mélanodispersion permanente. Gen. Comp. Physiol. 3: 26-45.

Mills, S.M. 1932a. Neurohumoral control of fish melanophore. Proc. Nat. Acad. Sci. 18: 540-543.

Mills, S.M. 1932b. Double innervation of melanophores. Proc. Nat. Acad. Sci. 18: 538-540.

Nagahama, H. 1953. Action of potassium ions on the melanophores in an isolated fish scale. Jap. J. Zool. 11(1): 75-85.

Nash, C.W., E.V. Luchka and K.H. Jhamandas. 1966. An investigation of sodium and calcium competition in vascular smooth muscle. Can. J. Physiol. Pharmacol. 44: 147-156.

Neill, R.M. 1940. On the existence of two types of chromatic behaviour in teleostean fishes. J. Exp. Biol. 17: 74-95.

Newman, H.H. 1918. Spawning behaviour and sexual dimorphism in Fundulus heteroclitus and allied fish. Contr. Zoo. Lab. Univ. Mich. No. 108: 314-349.

Newth, D.R. 1951. Experiments on the neural crest of the lamprey larva. J. Exptl. Biol. 28(3): 247-260.

Nickerson, M. 1949. The pharmacology of adrenergic blockade. Pharmacol. Rev. 1: 27-101.

Nickerson, M. 1959. Blockade of the actions of adrenaline and noradrenaline. in Symposium on Catechol Amines. The Williams and Wilkins Co. 1959.

Novales, R.R. 1959. The effects of osmotic pressure and sodium concentration on the response of melanophores to intermedin. Physiol. Zool. 32: 15-28.

Novales, R.R. 1962. The role of ionic factors in hormone action on the vertebrate melanophore. Amer. Zool. 2(3): 337-352.

Okun, M.R. 1965. Histogenesis of melanocytes. J. Invest. Dermatol. 44(5): 285-299.

Orton, G.L. 1953. Development and migration of pigment cells in some teleost fishes. J. Morphol. 93: 69-99.

Osborn, C.M. 1938. The role of the melanophore-dispersing principle of the pituitary in the color change of the catfish. J. Exp. Zool. 79: 309-330.

Osborn, C.M. 1939. The physiology of color change in flatfishes. J. Exp. Zool. 81: 479-515.

Osborn, C.M. 1941. Studies on the growth of integumentary pigment in lower vertebrates. II. The role of the hypophysis in melanogenesis in the common catfish (Ameiurus melas) Biol. Bull. 81: 352-363.

Palay, S.L. 1953. A note on the effects of hypophysectomy on the preoptico-hypophysial pathway in Fundulus. Bull. Bingham. Oceanogr. Coll. 17(2): 42-45.

Parker, G.H. 1934a. Acetyl choline and chromatophores. Proc. Nat. Acad. Sci. 20: 596-599.

Parker, G.H. 1934b. Neurohumours as activating agents for fish melanophores. Proc. Am. Phil. Soc. 74: 177-185.

Parker, G.H. 1934c. The prolonged activity of momentarily stimulated nerves. Proc. Nat. Acad. Sci., Wash., 20: 306-310.

Parker, G.H. 1935a. The chromatophoral neurohumors of the dogfish. J. Gen. Physiol. 18: 837-846.

Parker, G.H. 1935b. What are the resting and the active states of chromatophores? Proc. Nat. Acad. Sci. 21: 286-292.

Parker, G.H. 1935c. The electric stimulation of the chromatophoral nerve fibres in the dogfish. Biol. Bull. 68: 1-3.

Parker, G.H. 1940. Neurohumors as chromatophore activators. Proc. Amer. Acad. Arts Sci. 73: 165-195.

Parker, G.H. 1941. The organization of the melanophore system in bony fishes. The Collecting Net 16(5): 81-93.

Parker, G.H. 1943a. Color changes in animals. Q. Rev. Biol. 18: 205-227.

Parker, G.H. 1943b. Methods of estimating the effects of melanophore changes in animal coloration. Biol. Bull. 84: 273-284.

Parker, G.H. 1948. Animal Colour Changes and their Neurohumors. Cambridge University Press.

Paton, W.D.M. and E.J. Zaimis. 1952. The methonium compounds. Pharmacol. Revs. 4: 219-253.

Pickford, G.E. 1959. Endocrinological studies on the euryhaline killifish, Fundulus heteroclitus (Linn.) Yale J. Biol. Med. 31: 341-362.

Pickford, G.E. and J.E. Atz. 1957. The Physiology of the Pituitary Glands of Fishes. N. Y. Zool. Soc., N.Y.

Pickford, G.E. and B. Kosto. 1957. Hormonal induction of melanogenesis in hypophysectomized killifish (Fundulus heteroclitus). Endocrinology 61: 177-196.

Pickford, G.E. and J.G. Phillips. 1959. Prolactin, a factor in promoting survival of hypophysectomized killifish in fresh water. Science 130: 454.

Pouchet, G. 1871a. Sur les rapides changements de coloration provoqués expérimentalement chez les poissons. C. R. Acad. Sci. (Paris) 72: 866-869.

Pouchet, G. 1871b. VIII. On the connection of nerves and chromoblasts. Month. J. Micros. Sci. Lond.: 285-290.

Pouchet, G. 1871c. Du rôle des nerfs dans les changements de coloration des poissons. J. Anat. Physiol. 8: 71-74.

Pouchet, G. 1874. Note sur l'influence de l'ablation des yeux sur la coloration de certaines espèces animales. J. Anat. Physiol. 10: 558-560.

Pouchet, G. 1876. Des changements de coloration sous l'influence des nerfs. J. Anat. Physiol. 12: 1-90, 113-165.

Pye, J.D. 1964. Nervous control of chromatophores in teleost fish I-IV. J. Exp. Biol. 41: 525-557.

Rahn, H. 1941. The pituitary regulation of melanophores in the rattlesnake. Biol. Bull. 80: 228-237.

Rangier, M. 1960. Sur la constitution chemique des mélanines. C. R. Acad. Sci. (Paris) 251(1): 1954-1955.

Rasquin, P. 1958. Studies in the control of pigment cells and light reactions in recent teleost fishes. Part I. Morphology of the pineal region. Part II. Reactions of the pigmentary system to hormonal stimulation. Bull. Am. Mus. Nat. Hist. 115: 1-68.

Rawles, M.E. 1948. Origin of melanophores and their role in development of color patterns in vertebrates. Physiol. Rev. 28: 383-408.

Reinhardt, W.O., I.I. Geschwind, J.O. Porath and C.H. Li. 1952. Significance of intermedin activity in adrenocorticotrophic hormone preparations. Proc. Soc. Exp. Biol. Med. 80: 439-442.

Redfield, A.C. 1916. The coordination of chromatophores by hormones. Science 43: 580-581.

Robinson, M.A. and G.T. Scott. 1960. Frog melanophore dispersing action of meprobamate. Bioch. and Biophys. Res. Comm. 2(1): 19-21.

Roggen, D. 1962. An objective melanophore index. Acta Anat. 49: 260-265.

Romer, A.S. 1963. The Vertebrate Body (3rd ed.). W. B. Saunders Co., Philadelphia.

Santamarina, E. 1958. Melanin pigmentation in bovine pineal gland and its possible correlation with gonadal function. Can. J. Biochem. Physiol. 36: 227-235.

Sawyer, W.H., R.A. Munsick and H.B. van Dyke. 1961. Pharmacological characteristics of the active principles in neurohypophysial extracts from several species of fish. Endocrinology 68: 215-225.

Schwartz, F.J. 1964. Personal communication.

Schwartz, F.J. 1966. Personal communication.

Scott, G.T. 1962. Physiological action of psychoactive drugs on melanocytes in fish and frogs. Psychopharmacol. Serv. Cent. Bull. 2(5): 58-63.

Silvers, W.K. 1961. Genes and the pigment cells of mammals. Science 134: 368-373.

Smith, P.E. 1916. Experimental ablation of the hypophysis in the frog embryo. Science 44: 280-282.

Smith, D.C. 1928. The effect of temperature on the melanophores of fish. J. Exp. Zool. 52: 183-234.

Smith, D.C. 1931. The action of certain autonomic drugs upon the pigmentary responses of Fundulus. J. Exp. Zool. 58: 423-453.

Smith, D.C. 1936. A method for recording chromatophore pulsations in isolated fish scales by means of a photoelectric cell. J. Cell. Comp. Physiol. 8: 83-87.

Spaeth, R.A. 1913. The physiology of the chromatophores of fishes. J. Exp. Zool. 15(4): 527-585.

Spaeth, R.A. 1916a. Evidence proving the melanophore to be a disguised type of smooth muscle cell. J. Exp. Zool. 20: 193-215.

Spaeth, R.A. 1916b. The responses of single melanophores to electrical stimulation. Am. J. Physiol. 41: 577-596.

Spaeth, R.A. and H.G. Barbour. 1917a. The responses of fish melanophores to sympathetic stimulants and depressants. J. Pharm. and Exptl. Ther. 9: 356-357.

Spaeth, R.A. and H.G. Barbour. 1917b. The action of epinephrine and ergotoxin upon single physiologically isolated cells. J. Pharm. and Exptl. Ther. 9: 431-440.

Steelman, S.L. and R. Guillemin. 1959. Adrenocorticotropic activity of α MSH. Proc. Soc. Exptl. Biol. Med. 101: 600-601.

Sulman, F.G. 1952. The effect of ACTH on the frog chromatophores. Acta. Endocrinol. 10: 320-332.

Sumner, F.B. 1935a. Evidence for the protective value of changeable coloration in fishes. Amer. Naturalist 69: 245-266.

Sumner, F.B. 1935b. Studies of protective color change. III. Experiments with fishes both as predators and prey. Proc. Nat. Acad. Sci. 21: 345-353.

Sumner, F.B. 1945. The cause must have had eyes. Sci. Month. 60: 181-186.

Swan, G.A. 1963. Chemical structure of melanins. Ann. N. Y. Acad. Sci. 100: 1005-1019.

Teague, R.S. and J.R. Patton. 1960. Analysis of the spectrophotometric reflectance response of frogs to melanophore hormone. J. Cell. Comp. Physiol. 56: 15-24.

Thing, E. 1952. Melanophore reaction and adrenocoticotrophic hormone. I. Comparison of methods based upon photoelectric measurements and microscopic observations of the melanophores. Acta. Endocrinol. 10: 295-319.

Toman, J.E. 1952. Neuropharmacology of peripheral nerve. Pharmacol. Revs. 4: 168-218.

Turner, W.J. and A. Carl. 1955. Effect of reserpine on the melanophores of fish. Science 121: 877-878.

Varon, H.H. 1959. MSH-like substance from human placenta. Proc. Soc. Exptl. Biol. Med. 100: 609-610.

van de Veerdonk, F.C.G. 1960. Serotonin, a melanocyte-stimulating component in the dorsal skin secretion of Xenopus laevis. Nature 187: 948-949.

Vilter, V. 1937. Recherches histologiques et physiologiques sur la fonction pigmentaire des sélaciens. Bull. Soc. Sci. Arachon. 34: 65-136.

Waring, H. 1963. The Colour Change Mechanisms of Cold-Blooded Vertebrates. Academic Press, N.Y.

Waring, H. and F.W. Landgrebe. 1949. The melanophore excitant properties of various pituitary glands. Austral. J. Exp. Biol. Med. Sci. 27: 331-336.

Waring, H. and F.W. Landgrebe. 1950. Hormones of the posterior pituitary in The Hormones, vol. II. G. Pincus and K. Thimann, eds. Academic Press, N.Y.

Weisel, G.F. 1948. Comparative action of the fish pituitary on chromatophores. Proc. Mont. Acad. Sci. 8: 56-58.

Weisel, G.F. 1950. The comparative effects of teleost and beef pituitary on chromatophores of cold-blooded vertebrates. Biol. Bull. 99: 487-496.

Weissbach, H., B.G. Redfield, and J. Axelrod. 1960. Biosynthesis of melatonin: enzymic conversion of serotonin to N-acetyl-serotonin. Biochem. et Biophys. Acta 43: 352-353.

Wilber, C.G. 1954. Pharmacological studies on the melanophores in Fundulus heteroclitus. Med. Lab. Res. Rep. 278: 1-9.

Wilber, C.G. 1958. Chromatic responses of Fundulus to lysergic acid. Am. J. Physiol. 194(3): 488-490.

Wilber, C.G. 1960. Pharmacological studies on the melanophores in Fundulus heteroclitus. Progr. Fish Cult. 22: 34-37.

Wright, P.A. 1954. A convenient and rapid technique for assay of intermedin. Papers Mich. Acad. Sci., Arts Leterrs. 34: 271-279.

Wright, M.R. and A.B. Lerner. 1960. On the movement of pigment granules in frog melanocytes. Endocrinology 66: 599-609.

Wurtman, R.J., J. Axelrod and J.E. Fischer. 1964. Melatonin synthesis in the pineal gland: Effect of light mediated by the sympathetic nervous system. Science 143: 1328-1329.

Wurtman, R.J., J. Axelrod and L.S. Phillips. 1963. Melatonin synthesis in the pineal gland: control by light. Science 142: 1071-1073.

Wyman, L.C. 1924a. Blood and nerve as controlling agents in the movements of melanophores. J. Exp. Zool. 39: 73-132.

Wyman, L.C. 1924b. The reactions of the melanophores of embryonic and larval Fundulus heteroclitus to certain chemical substances. J. Exp. Zool. 40: 161-180.

Young, R.T. 1916. Some experiments on protective coloration. J. Exp. Zool. 20: 457-500.

Young, J.Z. 1935. The photoreceptors of lampreys. II. The functions of the pineal complex. J. Exp. Biol. 12: 254-270.

Young, J.Z. and C.W. Bellerby. 1935. The response of the lamprey to injection of anterior lobe pituitary extract. J. Exp. Biol. 12: 246-253.

Zimmerman, S.B. and H.C. Dalton. 1961. Physiological responses of amphibian melanophores. Physiol. Zool. 34: 21-33.

APPENDIX I: LIST OF DRUGS

Drugs are listed in the order that they appear in the Tables.
(data from Goodman and Gilman 3rd ed. and Merck Index 7th ed.)

Adrenaline: 1-β-(3, 4-dihydroxyphenyl)-α-methylamino-ethanol

Epinephrine hydrochloride USP, 1:1000; Parke, Davis Co.

l-epinephrine bitartrate; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Noradrenaline: 1-2-amino-1-(3, 4-dihydroxyphenyl)-ethanol. (l-arterenol, l-norepinephrine, l-noradrenaline)

l-Noradrenaline bitartrate, ("Levophed") Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Levarterenol bitartrate injection USP; Winthrop Laboratories of Canada, Aurora, Ontario

Ephedrine: α-(1-methylaminoethyl) benzyl alcohol. (l-Ephedrine)

Ephedrine hydrochloride NF; University of Alberta Hospital

Ephedrine sulfate USP; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Tyramine: 4-Hydroxyphenethylamine

Tyramine hydrochloride; Leitz

Dopamine: 3, 4-dihydroxyphenylethylamine

Dopamine hydrochloride; Mann Research Laboratories Inc., New York, U.S.A.

Tryptamine: 3-(2-Aminoethyl) indole

Tryptamine hydrochloride; Light

Isoprenaline: dl-β-3,[4-dihydroxyphenyl]-α-isopropylaminoethanol.
(isoproterenol, isopropylarterenol, isopropyl-
noradrenaline, isopropylnorepinephrine)

Isoprenaline; University of Alberta Hospital

Amphetamine: dl-β-phenylisopropylamine. (benzedrine)

D Amphetamine hydrochloride; Nutritional Biochemicals
Corp., Cleveland, Ohio, U.S.A.

dl-Amphetamine sulfate; Light

Dibenamine: N, N-dibenzyl-β-chloroethylamine.

Dibenamine hydrochloride; gift

Dibenzyline: N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl) benzylamine
(Dibenyline) Phenoxybenzamine hydrochloride; Smith, Kline
and French Co., Montreal, Quebec

Tolazoline: 2-Benzyl-2-imidazoline. (Priscoline)

Tolazoline hydrochloride injection USP ("Priscoline");
Ciba, Dorval, Quebec

Regitin: 2-[N'-p-tolyl-N'(m-hydroxyphenyl)-aminoethyl]
imidazoline (Phentolamine, Regitine, Rogetine)

Regitin Methansulfonat; Ciba, Dorval, Quebec

Yohimbine: $C_{21}H_{26}N_2O_3$

Yohimbin hydrochlorid BPC; British Drug Houses Ltd., London

Hydrogenated ergot derivatives: Dihydroergocornine, dihydroergocristine and dihydroergokryptine as methanesulfonates.

"Hydergine"; Sandoz Ltd., Basle

Ergotamine bitartrate: $(C_{33}H_{35}N_2O_5)_2 \cdot H_2C_4H_4O_6$

(Femergin, Gynergen)

Ergotamine bitartrate; Mann Research Laboratories Inc., New York, U.S.A.

Propanalol: 1-isopropylamino-3-(1-naphthyloxy) propan-2-ol hydrochloride (Inderal)

Propanalol; Ayerst Laboratories, St. Laurent, Quebec

Acetylcholine: Acetyl choline chloride; Mann Research Laboratories Inc., New York, U.S.A.

"Acéoline"; Lab. Welcker and Cie Ltée, Montreal, Quebec

Eserine: $(C_{15}H_{21}N_3O_2)_2 \cdot H_2SO_4$

Eserine sulfate USP VII; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Physostigmine sulfate; Merck and Co., Rahway, N.J., U.S.A.

Carbachol: (2-hydroxethyl) trimethyl ammonium chloride carbamate. (Carbamylcholine chloride, choline chloride carbamate)

Carbamylcholine chloride; Mann Research Laboratories Inc., New York, U.S.A.

Carbachol injection BP; British Drug Houses Ltd., London

Mecholyl: Acetyl-β-methylcholine chloride (Mecholyl chloride, Mecholin)

Methacholine chloride NF; Merck and Co., Rahway, N.J., U.S.A.

Nicotine: 1-Methyl-2-(3-pyridyl) pyrrolidine.

"Black Leaf 40", 40% aqueous solution of nicotine sulfate; Diamond Black Leaf Co., Cleveland Ohio, U.S.A.

Hexamethonium: Hexamethylenebis trimethyl-ammonium chloride

Hexamethonium chloride; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Hexamethonium bitartrate; Light

Pentolinium: 1, 1'-Pentamethylenebis [1-methylpyrrolidinium hydrogen tartrate] (Pentolinium tartrate, Ansolysen)

Pentolinium tartrate; Poulenc Co., Montreal, Quebec

Presidal: Pentacynium bis [methyl sulfate]

"Presidal"; The Wellcome Research Laboratories, Beckenham, England

Bretylium: (o-bromobenzyl) ethyldimethylammonium p-toluenesulfonate (Darenthin)

Bretylium tosylate; Wellcome Research Laboratories, Beckenham, England

Guanethidine: [2-(octahydro-1-azocinyl)-ethyl] guanidine sulfate. (Ismelin)

"Ismelin", Ciba, Horsham, Essex, England

Atropine: dl-Hyoscyamine.

Atropine sulfate· H_2O; Mann Research Laboratories Inc., New York, U.S.A.

Atropine sulfate; Merck and Co., Rahway, N.J., U.S.A.

Parnate: Tranylcypromine

Parnate; Smith,Kline and French, Montreal, Quebec

Pargyline: N-Benzyl-N-Methyl-2-propynlamine HCl.

(Eutonyl)

Pargyline; Abbott Laboratories, Montreal, Quebec

Serotonin: 3-(2-Aminoethyl)-5-indolol.(5-hydroxytryptamine, enteramine)

5-hydoxytryptamine creatinine sulfate; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

5-hydroxytryptamine phosphate; Light

3-OH Tyramine: 3-OH Tyramine; Leitz

5-OCH_3 Tryptamine: 5-Methoxy Tryptamine; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Melatonin: N-Acetyl-5-Methoxytryptamine

N-Acetyl-5-Methoxytryptamine; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Reserpine: 3, 4, 5 - Trimethoxybenzoyl methyl reserpate

"Serpasil" phosphate (lyophillized); CIBA Co. Ltd., Dorval, Quebec

"Serpasil" Reserpine injection USP; CIBA Co. Ltd., Dorval, Quebec

Reserpine; Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Posterior Pituitary Powder: Pituitary powder, posterior USP; Mann Research Laboratories Inc., New York, U.S.A.

Chlorpromazine: 2-Chloro-10-(3-dimethylaminopropyl)-phenothiazine
(Largactil, Thorazine)
Chlorpromazine HCl; Poulenc Co., Montreal, Quebec

Histamine: 2-(4-imidazolyl)-ethylamine
Histamine di Phosphate USP (m.p. 140° C); Mann Research Laboratories Inc., New York, U.S.A.
Histamine di Phosphate (PH) (m.p. 127°-132° C); Mann Research Laboratories Inc., New York, U.S.A.

Substances Used to Treat Fish and to Prepare Them for Experiments

Hyamine: 50% aqueous solution of methyl dodecyl benzyl trimethyl ammonium chloride and methyl dodecyl xylylene bis (trimethyl ammonium chloride)
"Hyamine 2389"; Rohm and Haas, Philadelphia, Pa., U.S.A.

Acriflavine: a mixture of 2, 8-diamino-10-methylacridinium chloride and 2, 8-diamino acridine
Acriflavine; K and K Laboratories, Plainsview, New York, U.S.A.

Malachite green: Fisher Scientific Co., Fairlawn, N.J., U.S.A.

Neptune Salts: Westchester Aquarium Supply Co., Inc., White Plains, N.Y., U.S.A.

Pyridyl mercuric acetate: K and K Laboratories, Plainsview, New York, U.S.A.

MS-222: Tricaine methanesulfonate
"MS-222"; Sandoz Pharmaceuticals, Dorval, Quebec

VII. APPENDIX II: SAMPLE EXPERIMENTAL PROTOCOLS AND RECORDS

Reproduced below are typical *in vivo* and *in vitro* experimental protocols and results, as well as a few comments indicating the interpretation of these results.

In vivo experiment

Protocol:

1. Four *Fundulus* taken from holding tank, and date and type of previous treatment, if any, recorded. Usually it was possible to select fish so that each fish of a pair was dissimilar either in size or sex, thus making it unnecessary to separate the two fish of a pair at this point. If readily distinguishable fish were not available it was necessary to separate them in numbered clear plastic boxes.
2. Fish adapted in pairs on white and black backgrounds for 15 minutes.
3. Tints of the fish expressed as a Derived Ostwald Index are recorded at the end of the adaptation period. These observations are shown opposite "Time 0" in the records below.
4. Fish given 100 mcg. carbachol in 0.1 ml by intra-peritoneal injection, and returned to their backgrounds.
5. Five minutes after the injection the tints were again recorded. This is noted opposite "Time 5" below. The tints were observed and recorded at 5-minute intervals for a further 25 minutes.

6. The fish were placed in numbered clear plastic boxes, if this had not been done previously, and kept in a holding tank overnight.
7. The tint and condition of the fish were noted next morning, and survivors were returned to a holding tank designated for fish receiving carbachol.

Colour Condition in Intact Fish as Recorded by Derived Ostwald Index

DRUG - Carbachol, 100 mcg. - no previous treatment

White Background			Black Background	
Time (mins.)	Box No. 1	Box No. 2	Box No. 3	Box No. 4
0	2.5	2.5	6.0	6.0
5	2.5	2.5	4.5 mottled	4.5 mottled
10	2.5	2.5 mottled to 4.5	5.0 "	5.0 "
15	2.5	2.5 "	5.5 "	5.5 in distress
20	2.5	4.5 " (some 2.5)	5.5 "	5.5 mottled in distress
25	2.5	4.5 "	5.5	6.0 " " "
30	2.5	4.5 "	5.5	6.5 " " "
over night	O.K. 3.0	Dead "	Dead	Dead

Comments: 1. The fish in Box No. 1 obviously did not receive an effective dose, probably because of leakage.

2. The fish in Box No. 2 showed dark mottling (DOI = 4.5) 10 minutes after injection. After a further 10 minutes the fish was predominantly dark, but a few light patches (DOI = 2.5) persisted throughout the observation period. Slight distress was evident.

3. The fish in Box No. 3 showed light patches (DOI = 4.5) five minutes after injection, but soon returned to a darker over-all tint (DOI = 5.5). Slight distress was evident.

4. The last fish (No. 4) showed a similar patchy paling which soon disappeared. Great distress was evident.

5. Because all but one of the fish showed some degree of distress which increased as time passed, it seemed probable that the dose was lethal or nearly so. Background exchanges were not attempted because experience showed that the responses of fish in distress for any reason were unreliable and consistent results could not be achieved under these circumstances.

6. Since all three fish evidencing a colour change as a result of the injection of 100 mcg of carbachol were dead the next morning, it was concluded that this dose was probably too high. The experiment was repeated with doses of 100, 10 and 1 mcg. Similar results were again obtained for the first two doses, and the injection of 1 mcg. of carbachol had no effect. These records are not shown.

In vitro experiment

Protocol: 1. Scales were removed from the mid-dorsal region of a Fundulus and placed in three small glass dishes containing saline. The composition of this saline is given in Materials and Methods.

2. The first two dishes were designated as experimental, the last as the control.

3. Mean Melanophore Indices (MMI) were calculated and recorded as noted in Materials and Methods. The Roman numerals from I to V in the records below refer to the five stages of the Hogben-Slome Melanophore Index. Readings were made with the aid of a binocular zoom dissecting microscope and the stage was illuminated from below only long enough to make the observations.

4. After steady MMI readings had been obtained for at least 10 minutes in all three dishes, the saline was removed by pipette from the experimental dishes and replaced with a solution of carbachol at a concentration of 1 mg/ml (arrow). The saline was also removed from the control dish at the same time, and replaced with fresh saline (arrow).

5. The MMI for each scale was read immediately and at five-minute intervals thereafter.

Scoring of Melanophores by Melanophore Index

#1 Experimental
DRUG - Carbachol - 1 mg/ml saline

Time	I	II	III	IV	V	MMI	Change	
0		111	1111111			2.7		S
5		11	1111111	1		2.9	0.2	A
10		1	11111111	1		3.0	0.1	L
15			no change			3.0	0	I
20			no change			3.0	0	N
25			no change			3.0	0	E
→ Drug introduced								
0		111111	1111			2.4	-0.6	
5		11111	11111			2.5	0.1	D
10			no change			2.5	0	R
15			no change			2.5	0	U
20			no change			2.5	0	G
25			no change			2.5	0	

#2 Experimental
DRUG - Carbachol - 1 mg/ml in saline

Time	I	II	III	IV	V	MMI	Change	
0	11	11111	11	1		2.2		S
5	1	11111111	11			2.1	-0.1	A
10		no change				2.1	0	L
15		no change				2.1	0	I
20		no change				2.1	0	N
25		no change				2.1	0	E
→	Drug introduced							
0	111	1111111				1.7	-0.4	
5	1111	111111				1.6	-0.1	D
10		no change				1.6	0	R
15		no change				1.6	0	U
20		no change				1.6	0	G
25		no change				1.6	0	

#3 Experimental
DRUG - Control for Carbachol - 1 mg/ml

Time	I	II	III	IV	V	MMI	Change	
0		1111111	111			2.0		S
5		111111	1111			2.4	0.4	A
10			no change			2.4	0	L
15			no change			2.4	0	I
20		11111	11111			2.5	0.1	N
25			no change			2.5	0	E
→ Saline changed								
0			no change			2.5	0	S
5		1111111	111			2.3	-0.2	A
10	111	11111	11			1.9	-0.4	L
15	11	1111111	1			1.9	0	I
20			no change			1.9	0	N
25			no change			1.9	0	E

Comments: 1. Steady MMI readings in saline were reached rapidly by the scales in the two experimental dishes, but not as quickly in the control. Steady readings usually were not reached at the same time in the three scales.

2. It will be noted that the MMI in saline rose slightly in the first experimental scale, fell very slightly in the second, and rose in the control. These changes gave steady MMI readings of 3.0, 2.1, and 2.5 respectively. No explanation can be advanced for this behaviour.

3. The change of MMI in No. 1 after addition of the drug solution was considered to have possible significance, and probably insignificant in No. 2. No change of MMI was noted when the saline in the control dish was changed (arrow). A slight transient increase of the MMI was a frequent observation following this procedure.

4. While the results reproduced here were taken into consideration in assessing the action of carbachol on melanophores, it was felt that the effect at a concentration of 1 mg/ml was not clear cut. The experiment was repeated at the same concentration. Lower concentrations of carbachol were also tested, in case the lack of a definite effect at the higher concentrations was due to self-blockade. The results in all cases were essentially similar: a very slight, transient aggregation, and this is indicated by A(?) in Table 3.

APPENDIX III: PHOTOGRAPHS OF A SET OF OSTWALD PAPERS USED IN THE EXPERIMENTS

As these are photographic reproductions of the Papers, the shades may not correspond exactly with the originals.

APPENDIX IV: DATA ON NUMBERS OF EXPERIMENTS

Drug	in vivo	in vitro	Caudal band experiments
Adrenaline	50	24	4
Noradrenaline	50	22	4
Isoprenaline	14		2
Dopamine	16	16	1
Tyramine	22	20	3
Ephedrine	38		3
Amphetamine	4		1
Tryptamine	10	14	1
Dibenamine	14	18	1
Dibenzyline	4	4	4
Tolazoline	16	4	
Regitin	28	24	2
"Hydergine"	14	4	5
Yohimbine	12		4
Propanalol	4		2
Bretylium	48	4	3
Guanethidine	16		4
Acetylcholine	150	12	4
Carbachol	54	18	4
Mecholyl	4		
Eserine	138	8	4
Acetylcholine and Eserine	150		1
Atropine	40	4	2
Nicotine	16	10	
Acetylcholine after Eserine and Atropine	27		
Hexamethonium	28	4	4
Pentolinium	8	4	
Presidal	36	4	3
Parnate	4	2	2
Pargyline	4		2
Serotonin (5HT)	16	22	3
5OCH_3 Tryptamine	12	8	1
3OH Tyramine	6	4	3
Histamine	4	4	1
Melatonin	20	34	1
Reserpine	18	4	3
Chlorpromazine	6	2	4
Posterior pituitary powder	8	4	1
Adrenaline after Tolazoline	8		
Propanalol	8		

Appendix IV cont.

Drug	in vivo	in vitro	Caudal band experiments
Adrenaline after			
Dibenamine	8		
"Hydergine"	4		
Presidal	8		
Bretylium	10		
Noradrenaline after			
Bretylium	6		
Hexamethonium	6		
Presidal	6		
Ephedrine after			
Bretylium	8		
Presidal	8		
Hexamethonium	6		
Reserpine after			
Parnate	4		
Pargyline	8		
Experiments with			
EDTA		5	
Light, heat and cold		35	
Controls (saline)	28	165	
MS-222 (immersion)	56		
Total change time	102		
Nerve and spinal sections	20		

APPENDIX V: pH OF SOME AGENTS IN SALINE AT A CONCENTRATION OF 1 MG/ML

Saline used as solvent: pH 6.7

Adrenaline bitartrate: pH 3.4

Noradrenaline bitartrate: pH 3.4

Tolazoline hydrochloride: pH 4.0

Acetyl choline chloride: pH 6.2

Eserine sulfate: pH 5.4

Hexamethonium chloride: pH 6.5

Bretylium tosylate: pH 6.5

Melatonin: pH 6.7

Measurements were made with a Beckman Model 72 pH meter.

VIII. FIGURE 1: THE MELANOPHORE INDEX

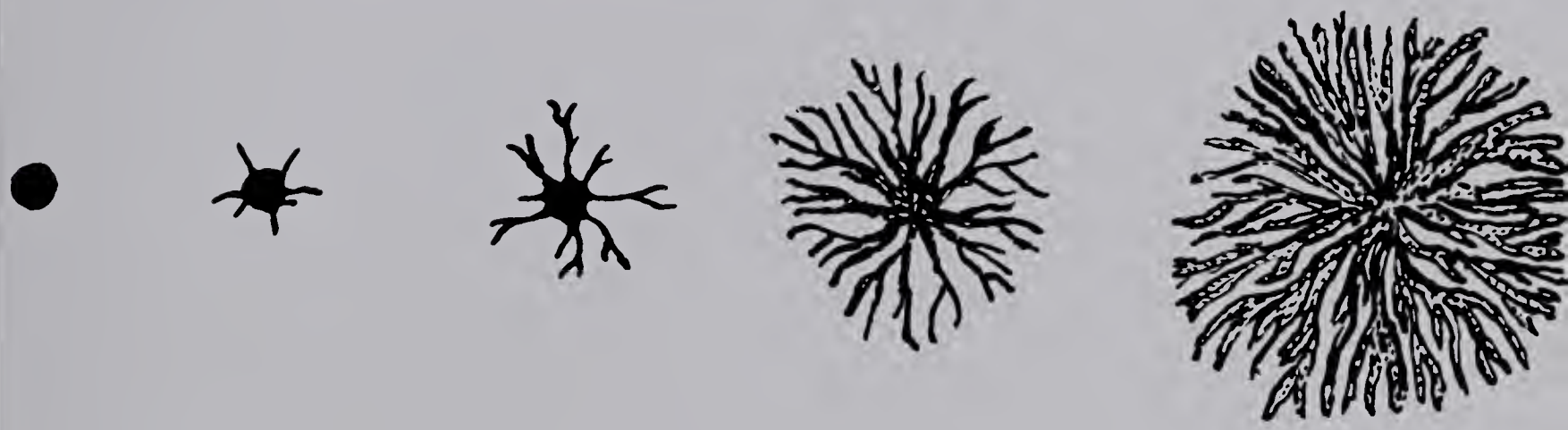

Fig. 1. The melanophore index.

From: Healey, E.G. 1951. The colour changes of the minnow (Phoxinus laevis Ag.). I. Effects of spinal section between vertebrae 5 and 12 on the responses of the melanophores. J. Exp. Biol. 28: 297-319.

M.I. = 1 shows melanophores in an aggregated condition, as in a maximally pale fish. M.I. = 5 is the melanophore in a dispersed condition, as in a maximally dark fish.